

[0502] Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

[0503] In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

[0504] Presence of the labeled molecule can be detected in the patient using methods known in the art for *in vivo* scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

[0505] In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Kits

[0506] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the

polypeptide of interest. In another specific embodiment, the kits of the present invention comprise two or more antibodies (monoclonal and/or polyclonal) that recognize the same and/or different sequences or regions of the polypeptide of the invention. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

[0507] In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

[0508] In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

[0509] In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody.

Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

[0510] In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

[0511] The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

[0512] Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface- bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

[0513] The invention further relates to antibodies which act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Included are both receptor-specific antibodies and ligand-specific antibodies. Included are receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. Also included are receptor-specific antibodies which both prevent ligand binding and receptor activation. Likewise, included are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby

preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included are antibodies which activate the receptor. These antibodies may act as agonists for either all or less than all of the biological activities affected by ligand-mediated receptor activation. The antibodies may be specified as agonists or antagonists for biological activities comprising specific activities disclosed herein. Further included are antibodies that bind to Neutrokin-alpha and/or Neutrokin-alphaSV irrespective of whether Neutrokin-alpha or Neutrokin-alphaSV is bound to a Neutrokin-alpha Receptor. These antibodies act as Neutrokin-alpha and/or Neutrokin-alphaSV agonists as reflected in an increase in cellular proliferation in response to binding of Neutrokin-alpha and/or Neutrokin-alphaSV to a Neutrokin-alpha receptor in the presence of these antibodies. The above antibody agonists can be made using methods known in the art. See e.g., WO 96/40281; US Patent 5,811,097; Deng, B. et al., Blood 92(6):1981-1988 (1998); Chen, Z. et al., Cancer Res. 58(16):3668-3678 (1998); Harrop, J.A. et al., J. Immunol. 161(4):1786-1794 (1998); Zhu, Z. et al., Cancer Res. 58(15):3209-3214 (1998); Yoon, D.Y. et al., J. Immunol. 160(7):3170-3179 (1998); Prat, M. et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard, V. et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard, J. et al., Cytokinde 9(4):233-241 (1997); Carlson, N.G. et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman, R.E. et al., Neuron 14(4):755-762 (1995); Muller, Y.A. et al., Structure 6(9):1153-1167 (1998); Bartunek, P. et al., Cytokine 8(1):14-20 (1996) (said references incorporated by reference in their entireties).

[0514] At least fourteen murine monoclonal antibodies have been generated against Neutrokin-alpha. These monoclonal antibodies are designated: 12D6, 2E5, 9B6, 1B8, 5F4, 9A5, 10G12, 11G12, 16B4, 3D4, 16C9, 13D5, 15C10, and 12C5. Preliminary analysis of these antibodies indicates that each binds Neutrokin-alpha protein in a Western blot analysis and when Neutrokin-alpha protein is bound to an ELISA plate. However, further analysis of antibodies 12D6, 2E5, 9B6, 1B8, 5F4, 9A5, 10G12, 11G12, and 16B4 indicates that only the antibodies designated 12D6, 9B6, 2E5, 10G12, 9A5, and 11G12 bind a membrane-bound form of Neutrokin-alpha. Thus, a subset of the monoclonal antibodies generated against Neutrokin-alpha have been determined to bind only the membrane-bound form of Neutrokin-alpha (i.e., this subset does not bind the soluble form of Neutrokin-alpha corresponding to amino acids 134 to 285 of SEQ ID

NO:2), which as discussed herein, is primarily limited to expression on monocytes and dendritic cells.

[0515] Antibody 9B6 has been found to bind specifically to the membrane – bound form of Neutrokin-alpha, but not to the soluble form of Neutrokin-alpha.

[0516] Epitope mapping of antibody 9B6 has indicated that this antibody binds specifically to an amino acid sequence contained in amino acid residues from about Ser-171 to about Phe-194 of SEQ ID NO:2. More particularly, epitope mapping has indicated that antibody 9B6 binds specifically to a peptide comprising amino acid residues Lys-173 to Lys-188 of SEQ ID NO:2.

[0517] In contrast, antibodies 16C9 and 15C10 have been found to bind the soluble form of Neutrokin-alpha (amino acids 134 to 285 of SEQ ID NO:2) and to inhibit Neutrokin-alpha-mediated proliferation of B cells. *See for example*, Example 10. The 15C10 antibody has also been found to inhibit binding of Neutrokin-alpha to its receptor. Epitope mapping of antibody 15C10 has indicated that this antibody binds specifically to an amino acid sequence contained in amino acid residues from about Glu-223 to about Tyr-246 of SEQ ID NO:2. More particularly, epitope mapping has indicated that antibody 15C10 binds specifically to a peptide comprising amino acid residues Val-227 to Asn-242 of SEQ ID NO:2. Antibody 15C10 also binds specifically to a peptide comprising amino acid residues Phe-230 to Cys-245 of SEQ ID NO:2. It is likely that the epitope of 15C10 is conformational rather than linear and that antibody 15C10 may make specific binding contacts with amino acid residues in the full length Neutrokin-alpha protein outside of amino acid residues 223-246 of SEQ ID NO:2 as well as within amino acid residues 223-246.

[0518] Furthermore, competitive binding studies have shown that antibodies 3D4 and 15C10 bind similar or identical epitopes (see Example 15).

[0519] As described above, anti-Neutrokin-alpha monoclonal antibodies have been prepared. Hybridomas producing the antibodies referred to as 9B6 and 15C10 were deposited with the ATCC located at 10801 University Boulevard, Manassas, Virginia 20110-2209, on January 27, 2000 and were assigned deposit accession numbers PTA-1158 and PTA-1159, respectively. The ATCC deposits were made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

[0520] NS0 cell lines engineered to secrete chimeric forms of antibodies 3D4 and 15C10 were deposited with the ATCC located at 10801 University Boulevard, Manassas, Virginia 20110-2209, on October 24, 2001 and were assigned deposit accession numbers PTA-3795 and PTA-3794, respectively. Chimeric antibodies 3D4 and 15C10 contain murine variable regions and human constant (IgG1 and kappa) regions. The ATCC deposits were made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

[0521] In one embodiment, the antibodies of the invention have one or more of the same biological characteristics as one or more of the antibodies secreted by the deposited cell lines (ATCC accession numbers PTA-1158, PTA-1159, PTA-3795 and PTA-3794). By "biological characteristics" is meant, the in vitro or in vivo activities or properties of the antibodies, such as, for example, the ability to bind to Neutrokin-alpha (e.g., the polypeptide of SEQ ID NO:2, the mature form of Neutrokin-alpha, the membrane-bound form of Neutrokin-alpha, the soluble form of Neutrokin-alpha (amino acids 134 to 285 of SEQ ID NO:2), and an antigenic and/or epitope region of Neutrokin-alpha), the ability to substantially block Neutrokin-alpha/Neutrokin-alpha receptor binding, or the ability to block Neutrokin-alpha mediated biological activity (e.g., stimulation of B cell proliferation and immunoglobulin production). Optionally, the antibodies of the invention will bind to the same epitope as at least one of the antibodies specifically referred to herein. Such epitope binding can be routinely determined using assays known in the art.

[0522] Thus, in one embodiment, the invention provides antibodies that specifically bind the membrane-bound form of Neutrokin-alpha and do not bind the soluble form of Neutrokin-alpha. These antibodies have uses which include, but are not limited to, as diagnostic probes for identifying and/or isolating monocyte lineages expressing the membrane bound form of Neutrokin-alpha. For example, the expression of the membrane bound form of Neutrokin-alpha is elevated on activated monocytes, and accordingly, antibodies encompassed by the invention may be used to detect and/or quantitate levels of activated monocytes. Additionally, antibodies that only bind the membrane bound form of Neutrokin-alpha may be used to target toxins to neoplastic, preneoplastic, and/or other cells that express the membrane bound form of Neutrokin-alpha (e.g., monocytes and dendritic cells).

[0523] In another embodiment, antibodies of the invention specifically bind only the soluble form of Neutrokinne-alpha (amino acids 134 to 285 of SEQ ID NO:2). These antibodies have uses which include, but are not limited to, uses such as diagnostic probes for assaying soluble Neutrokinne-alpha in biological samples, and as therapeutic agents that target toxins to cells expressing Neutrokinne-alpha receptors (e.g., B cells), and/or to reduce or block in vitro or in vivo Neutrokinne-alpha mediated biological activity (e.g., stimulation of B cell proliferation and/or immunoglobulin production).

[0524] The invention also provides for antibodies that specifically bind both the membrane-bound and soluble form of Neutrokinne-alpha.

[0525] As described above, the invention encompasses antibodies that inhibit or reduce the ability of Neutrokinne-alpha and/or Neutrokinne-alphaSV to bind Neutrokinne-alpha receptor and/or Neutrokinne-alphaSV receptor in vitro and/or in vivo. In a specific embodiment, antibodies of the invention inhibit or reduce the ability of Neutrokinne-alpha and/or Neutrokinne-alphaSV to bind Neutrokinne-alpha receptor and/or Neutrokinne-alphaSV receptor in vitro. In another nonexclusive specific embodiment, antibodies of the invention inhibit or reduce the ability of Neutrokinne-alpha and/or Neutrokinne-alphaSV to bind Neutrokinne-alpha receptor and/or Neutrokinne-alphaSV receptor in vivo. Such inhibition can be assayed using techniques described herein or otherwise known in the art.

[0526] The invention also encompasses, antibodies that bind specifically to Neutrokinne-alpha and/or Neutrokinne-alphaSV, but do not inhibit the ability of Neutrokinne-alpha and/or Neutrokinne-alphaSV to bind Neutrokinne-alpha receptor and/or Neutrokinne-alphaSV receptor in vitro and/or in vivo. In a specific embodiment, antibodies of the invention do not inhibit or reduce the ability of Neutrokinne-alpha and/or Neutrokinne-alphaSV to bind Neutrokinne-alpha receptor and/or Neutrokinne-alphaSV receptor in vitro. In another nonexclusive specific embodiment, antibodies of the invention do not inhibit or reduce the ability of Neutrokinne-alpha and/or Neutrokinne-alphaSV to bind Neutrokinne-alpha receptor and/or Neutrokinne-alphaSV receptor in vivo.

[0527] As described above, the invention encompasses antibodies that inhibit or reduce a Neutrokinne-alpha and/or Neutrokinne-alphaSV-mediated biological activity in vitro and/or in vivo. In a specific embodiment, antibodies of the invention inhibit or reduce Neutrokinne-alpha- and/or Neutrokinne-alphaSV-mediated B cell proliferation in vitro. Such inhibition can be assayed by routinely modifying B cell proliferation assays

described herein or otherwise known in the art. In another nonexclusive specific embodiment, antibodies of the invention inhibit or reduce Neutrokin-alpha- and/or Neutrokin-alphaSV-mediated B cell proliferation in vivo. In a specific embodiment, the antibody of the invention is 15C10, or a humanized form thereof. In another preferred specific embodiment, the antibody is 16C9, or a humanized form thereof. Thus, in specific embodiments of the invention, a 16C9 and/or 15C10 antibody, or humanized forms thereof, are used to bind soluble Neutrokin-alpha and/or Neutrokin-alphaSV and/or agonists and/or antagonists thereof and thereby inhibit (either partially or completely) B cell proliferation.

[0528] Alternatively, the invention also encompasses, antibodies that bind specifically to a Neutrokin-alpha and/or Neutrokin-alphaSV, but do not inhibit or reduce a Neutrokin-alpha and/or Neutrokin-alphaSV-mediated biological activity in vitro and/or in vivo (e.g., stimulation of B cell proliferation). In a specific embodiment, antibodies of the invention do not inhibit or reduce a Neutrokin-alpha and/or Neutrokin-alphaSV-mediated biological activity in vitro. In another non-exclusive embodiment, antibodies of the invention do not inhibit or reduce a Neutrokin-alpha and/or Neutrokin-alphaSV mediated biological activity in vivo. In a specific embodiment, the antibody of the invention is 9B6, or a humanized form thereof.

[0529] As described above, the invention encompasses antibodies that specifically bind to the same epitope as at least one of the antibodies specifically referred to herein, in vitro and/or in vivo.

[0530] In a specific embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from about Ser-171 to about Phe-194 of SEQ ID NO:2, in vitro. In another specific, non-exclusive embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from about Ser-171 to about Phe-194 of SEQ ID NO:2, in vivo. In another specific, non-exclusive embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from Lys-173 to Lys-188 of SEQ ID NO:2, in vitro. In another specific, non-exclusive embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from Lys-173 to Lys-188 of SEQ ID NO:2, in vivo.

[0531] In an additional specific embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from about Glu-223 to about Tyr-246 of SEQ ID NO:2, in vitro. In another specific, non-exclusive embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from about Glu-223 to about Tyr-246 of SEQ ID NO:2, in vivo. In another specific, non-exclusive embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from Val-227 to Asn-242 of SEQ ID NO:2, in vitro. In another specific, non-exclusive embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from Val-227 to Asn-242 of SEQ ID NO:2, in vivo. In another specific, non-exclusive embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from Phe-230 to Cys-245 of SEQ ID NO:2, in vitro. In another specific, non-exclusive embodiment, the antibodies of the invention specifically bind to an amino acid sequence contained in amino acid residues from Phe-230 to Cys-245 of SEQ ID NO:2, in vivo.

[0532] The invention also provides antibodies that competitively inhibit the binding of the 9B6 monoclonal antibody produced by the hybridoma deposited as PTA-1159 to a polypeptide of the invention, preferably the polypeptide of SEQ ID NO:2, more preferable to a polypeptide having the amino acid sequence of residues Ser-171 to Phe-194 of SEQ ID NO:2. Competitive inhibition can be determined by any method known in the art, for example, using the competitive binding assays described herein. In preferred embodiments, the antibody competitively inhibits the binding of 9B6 monoclonal antibody by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, to the polypeptide of SEQ ID NO:2, or more preferable to a polypeptide having the amino acid sequence of residues Ser-171 to Phe-194 of SEQ ID NO:2.

[0533] The invention also provides antibodies that competitively inhibit the binding of the 15C10 monoclonal antibody produced by the hybridoma deposited as PTA-1158 to a polypeptide of the invention, preferably the polypeptide of SEQ ID NO:2, more preferable to a polypeptide having the amino acid sequence of residues Glu-223 to Tyr-246 of SEQ ID NO:2. In preferred embodiments, the antibody competitively inhibits the binding of 15C10 monoclonal antibody by at least 95%, at least 90%, at least 85%, at least 80%, at

least 75%, at least 70%, at least 60%, at least 50%, to the polypeptide of SEQ ID NO:2, or more preferable to a polypeptide having the amino acid sequence of residues Glu-223 to Tyr-246 of SEQ ID NO:2.

[0534] Additional embodiments of the invention are directed to the 9B6 antibody and to the hybridoma cell line expressing this antibody. A hybridoma cell line expressing Antibody 9B6 was deposited with the ATCC on January 7, 2000 and has been assigned ATCC Deposit No. PTA-1159. In a preferred embodiment, antibody 9B6 is humanized.

[0535] Additional embodiments of the invention are directed to the 15C10 antibody and to the hybridoma cell line expressing this antibody. A hybridoma cell line expressing Antibody 15C10 was deposited with the ATCC on January 7, 2000 and has been assigned ATCC Deposit No. PTA-1158. In a preferred embodiment, antibody 15C10 is humanized.

[0536] In a specific embodiment, the specific antibodies described above are humanized using techniques described herein or otherwise known in the art and then used as therapeutics as described herein.

[0537] In another specific embodiment, any of the antibodies listed above are used in a soluble form.

[0538] In another specific embodiment, any of the antibodies listed above are conjugated to a toxin or a label (as described infra). Such conjugated antibodies are used to kill a particular population of cells or to quantitate a particular population of cells. In a preferred embodiment, such conjugated antibodies are used to kill B cells expressing Neutrokin-alpha receptor on their surface. In another preferred embodiment, such conjugated antibodies are used to quantitate B cells expressing Neutrokin-alpha receptor on their surface. In another preferred embodiment, such conjugated antibodies are used to kill monocyte cells expressing the membrane-bound form of Neutrokin-alpha. In another preferred embodiment, such conjugated antibodies are used to quantitate monocyte cells expressing the membrane-bound form of Neutrokin-alpha and/or Neutrokin-alphaSV. In highly preferred embodiments, such conjugated antibodies that bind the membrane bound form of Neutrokin-alpha and/or Neutrokin-alphaSV are used to kill Acute Myleogenous Leukemia cells, Chronic Lymphocytic leukemia cells, Multiple Myeloma cells, Non-Hodgkin's Lymphoma cells, and Hodgkin's lymphoma cells.

[0539] The antibodies of the invention also have uses as therapeutics and/or prophylactics which include, but are not limited to, in activating monocytes or blocking monocyte activation and/or killing monocyte lineages that express the membrane bound form of Neutrokin-alpha on their cell surfaces (e.g., to treat, prevent, and/or diagnose myeloid leukemias, monocyte based leukemias and lymphomas, monocytosis, monocytopenia, rheumatoid arthritis, and other diseases or conditions associated with activated monocytes). In a specific embodiment, the antibodies of the invention fix complement. In other specific embodiments, as further described herein, the antibodies of the invention (or fragments thereof) are associated with heterologous polypeptides or nucleic acids (e.g. toxins, such as, compounds that bind and activate endogenous cytotoxic effector systems, and radioisotopes; and cytotoxic prodrugs).

[0540] In another embodiment, one or more monoclonal antibodies are produced wherein they recognize or bind Neutrokin-alpha and/or a mutein thereof, but do not recognize or bind Neutrokin-alphaSV and/or a mutein thereof. In a related embodiment, one or more monoclonal antibodies are produced wherein they recognize or bind Neutrokin-alphaSV and/or a mutein thereof, but do not recognize or bind Neutrokin-alpha and/or a mutein thereof.

[0541] As discussed above, antibodies to the Neutrokin-alpha and/or Neutrokin-alpha SV polypeptides of the invention can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" the Neutrokin-alpha, using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444 (1989), and Nissinoff, *J. Immunol.* 147(8):2429-2438 (1991)). For example, antibodies which bind to Neutrokin-alpha and/or Neutrokin-alpha SV and competitively inhibit the Neutrokin-alpha and/or Neutrokin-alpha SV multimerization and/or binding to ligand can be used to generate anti-idiotypes that "mimic" the Neutrokin-alpha TNF multimerization and/or binding domain and, as a consequence, bind to and neutralize Neutrokin-alpha or Neutrokin-alpha SV and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize Neutrokin-alpha ligand. For example, such anti-idiotypic antibodies can be used to bind Neutrokin-alpha and/or Neutrokin-alpha SV, or to bind Neutrokin-alpha and/or Neutrokin-alpha SV receptors on the surface of cells of B cell lineage, and thereby

block Neutrokin-alpha and/or Neutrokin-alpha SV mediated B cell activation, proliferation, and/or differentiation.

Immune System-Related Disorder Diagnosis

[0542] Neutrokin-alpha is expressed in kidney, lung, peripheral leukocyte, bone marrow, T cell lymphoma, B cell lymphoma, activated T cells, stomach cancer, smooth muscle, macrophages, and cord blood tissue, and particularly cells of monocytic lineage. Moreover, Neutrokin-alphaSV is expressed in primary dendritic cells. Additionally, Neutrokin-alpha is expressed on the cell surface of the following non-hematopoietic tumor cell lines. Colon carcinomas HCT 116 (ATCC Accession No. CCL-247) and HT-29 (ATCC Accession No. HTB-38); Colon adenocarcinomas Caco-2 (ATCC Accession No. HTB-37), COLO 201 (ATCC Accession No. CCL-224), and WiDr (ATCC Accession No. CCL-218); Breast adenocarcinoma MDA-MB-231 (ATCC Accession No. HTB-26); Bladder squamous carcinoma SCaBER (ATCC Accession No. HTB-3); Bladder carcinoma HT-1197 (ATCC Accession No. CRL-1473); Kidney carcinomas A-498 (ATCC Accession No. HTB-44), Caki-1 (ATCC Accession No. HTB-46), and Caki-2 (ATCC Accession No. HTG-47); Kidney, Wilms tumor SK-NEP-1 (ATCC Accession No. HTB-48); and Pancreas carcinomas Hs 766T (ATCC Accession No. HTB-134), MIA PaCa-2 (ATCC Accession No. CRL-1420), and SU.86.86 (ATCC Accession No. CRL-1837). For a number of immune system-related disorders, substantially altered (increased or decreased) levels of Neutrokin-alpha and/or Neutrokin-alphaSV gene expression can be detected in immune system tissue or other cells or bodily fluids (e.g., sera, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" Neutrokin-alpha and/or Neutrokin-alphaSV gene expression level, that is, the Neutrokin-alpha and/or Neutrokin-alphaSV expression level in immune system tissues or bodily fluids from an individual not having the immune system disorder. Thus, the invention provides a diagnostic method useful during diagnosis of an immune system disorder, which involves measuring the expression level of the gene encoding the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard Neutrokin-alpha and/or Neutrokin-alphaSV gene expression level, whereby an increase or decrease in the gene expression level compared

to the standard is indicative of an immune system disorder or normal activation, proliferation, differentiation, and/or death.

[0543] In particular, it is believed that certain tissues in mammals with cancer of cells or tissue of the immune system express significantly enhanced or reduced levels of the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide and mRNA encoding the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide when compared to a corresponding "standard" level. Further, it is believed that enhanced or depressed levels of the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide can be detected in certain body fluids (e.g., sera, plasma, urine, and spinal fluid) or cells or tissue from mammals with such a cancer when compared to sera from mammals of the same species not having the cancer.

[0544] For example, as disclosed herein, Neutrokin-alpha is highly expressed in cells of monocytic lineage. Accordingly, polynucleotides of the invention (e.g., polynucleotide sequences complementary to all or a portion of Neutrokin-alpha mRNA and/or Neutrokin-alphaSV mRNA) and antibodies (and antibody fragments) directed against the polypeptides of the invention may be used to quantitate or qualitate concentrations of cells of monocytic lineage (e.g., monocytic leukemia cells) expressing Neutrokin-alpha on their cell surfaces. These antibodies additionally have diagnostic applications in detecting abnormalities in the level of Neutrokin-alpha gene expression, or abnormalities in the structure and/or temporal, tissue, cellular, or subcellular location of Neutrokin-alpha and/or Neutrokin-alphaSV. These diagnostic assays may be performed in vivo or in vitro, such as, for example, on blood samples, biopsy tissue or autopsy tissue.

[0545] Additionally, as disclosed herein, Neutrokin-alpha receptor is expressed primarily on cells of B cell lineage. Accordingly, Neutrokin-alpha polypeptides of the invention (including labeled Neutrokin-alpha polypeptides and Neutrokin-alpha fusion proteins), and anti-Neutrokin-alpha antibodies (including anti-Neutrokin-alpha antibody fragments) against the polypeptides of the invention may be used to quantitate or qualitate concentrations of cells of B cell lineage (e.g., B cell related leukemias or lymphomas) expressing Neutrokin-alpha receptor on their cell surfaces.

[0546] Neutrokin-alpha polypeptides and antibodies additionally have diagnostic applications in detecting abnormalities in the level of Neutrokin-alpha receptor gene expression (e.g., transmembrane activator and CAML interactor (TACI, GenBank

accesion number AAC51790), and B-cell maturation antigen (BCMA, GenBank accession number NP_001183)), or abnormalities in the structure and/or temporal, tissue, cellular, or subcellular location of Neutrokin-alpha receptor and/or diagnosing activity/defects in signalling pathways associated with Neutrokin-alpha. These diagnostic assays may be performed in vivo or in vitro, such as, for example, on blood samples or biopsy tissue using techniques described herein or otherwise known in the art.

[0547] In one embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides or Neutrokin-alpha and/or Neutrokin-alphaSV agonists or antagonists (e.g., anti-Neutrokin-alpha and/or anti-Neutrokin-alphaSV antibodies) of the invention are used to treat, prevent, diagnose, or prognose an individual having an immunodeficiency.

[0548] Immunodeficiencies that may be treated, prevented, diagnosed, and/or prognosed with the Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides or Neutrokin-alpha and/or Neutrokin-alphaSV agonists or antagonists (e.g., anti-Neutrokin-alpha and/or anti-Neutrokin-alphaSV antibodies) of the invention, include, but are not limited to one or more immunodeficiencies selected from: severe combined immunodeficiency (SCID)-X linked, SCID-autosomal, adenosine deaminase deficiency (ADA deficiency), X-linked agammaglobulinemia (XLA), Bruton's disease, congenital agammaglobulinemia, X-linked infantile agammaglobulinemia, acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, unspecified hypogammaglobulinemia, agammaglobulinemia, common variable immunodeficiency (CVID) (acquired), chronic granulomatous disease, Wiskott-Aldrich Syndrome (WAS), X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, selective IgA deficiency, IgG subclass deficiency (with or without IgA deficiency), antibody deficiency with normal or elevated IgS, immunodeficiency with thymoma, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), selective IgM immunodeficiency, recessive agammaglobulinemia (Swiss type), reticular dysgenesis, neonatal neutropenia, severe congenital leukopenia, thymic alymphoplasia-aplasia or dysplasia with immunodeficiency, ataxia-telangiectasia, short limbed dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome-combined immunodeficiency with IgS, purine nucleoside

phosphorylase deficiency (PNP), MHC Class II deficiency (Bare Lymphocyte Syndrome) and severe combined immunodeficiency.

[0549] According to this embodiment, an individual having an immunodeficiency expresses aberrantly low levels of Neutrokin-alpha and/or Neutrokin-alpha SV when compared to an individual not having an immunodeficiency. Any means described herein or otherwise known in the art may be applied to detect Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention (e.g., FACS analysis or ELISA detection of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention and hybridization or PCR detection of Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides of the invention) and to determine the expression profile of Neutrokin-alpha and/or Neutrokin-alphaSV, polynucleotides and/or polypeptides of the invention in a biological sample.

[0550] A biological sample of a person afflicted with an immunodeficiency is characterized by low levels of expression of Neutrokin-alpha and/or Neutrokin-alphaSV when compared to that observed in individuals not having an immunodeficiency. Thus, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides and/or polypeptides of the invention, and/or agonists or antagonists thereof, may be used according to the methods of the invention in the diagnosis and/or prognosis of an immunodeficiency. For example, a biological sample obtained from a person suspected of being afflicted with an immunodeficiency ("the subject") may be analyzed for the relative expression level(s) of Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides and/or polypeptides of the invention. The expression level(s) of one or more of these molecules of the invention is (are) then compared to the expression level(s) of the same molecules of the invention as expressed in a person known not to be afflicted with an immunodeficiency. A significant difference in expression level(s) of Neutrokin-alpha, and/or Neutrokin-alphaSV, polynucleotides and/or polypeptides of the invention, and/or agonists and/or antagonists thereof, between samples obtained from the subject and the control suggests that the subject is afflicted with an immunodeficiency.

[0551] In another embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides or Neutrokin-alpha and/or Neutrokin-alphaSV agonists or antagonists (e.g., anti-Neutrokin-alpha and/or anti-Neutrokin-alphaSV antibodies) of the invention are used to treat, diagnose and/or prognose an individual having common

variable immunodeficiency disease ("CVID"; also known as "acquired agammaglobulinemia" and "acquired hypogammaglobulinemia") or a subset of this disease. According to this embodiment, an individual having CVID or a subset of individuals having CVID expresses aberrant levels of Neutrokin-alpha and/or Neutrokin-alpha Receptor on their B cells and/or monocytes, when compared to individuals not having CVID. Any means described herein or otherwise known in the art may be applied to detect Neutrokin-alpha polynucleotides or polypeptides of the invention and/or Neutrokin-alpha Receptor polypeptides (e.g., FACS analysis or ELISA detection of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention and hybridization or PCR detection of Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides of the invention) and to determine differentially the expression profile of Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention and/or Neutrokin-alpha receptor polypeptides in a sample containing at least monocyte cells or some component thereof (e.g., RNA) as compared to a sample containing at least B cells or a component thereof (e.g., RNA). In the instance where a sample containing at least monocyte cells or some component thereof (e.g., RNA) is determined to reflect Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotide or polypeptide expression and a sample containing at least B cells or a component thereof (e.g., RNA) is determined to reflect less than normal levels of Neutrokin-alpha receptor polynucleotide or polypeptide expression, the samples may be correlated with the occurrence of CVID (i.e., "acquired agammaglobulinemia" or "acquired hypogammaglobulinemia").

[0552] A subset of persons afflicted with CVID are characterized by high levels of expression of both Neutrokin-alpha and the Neutrokin-alpha receptor ("NAR") in peripheral or circulating B cells when compared to that observed in individuals not having CVID. In contrast, persons who are not afflicted with CVID are typically characterized by low levels of Neutrokin-alpha expression and high levels of NAR expression in peripheral or circulating B cells. Thus, Neutrokin-alpha, Neutrokin-alphaSV polypeptides, and/or NAR polypeptides, polynucleotides and/or polypeptides of the invention, and/or agonists or antagonists thereof, may be used according to the methods of the invention in the differential diagnosis of this subset of CVID. For example, a sample of peripheral B cells obtained from a person suspected of being afflicted with CVID ("the

subject") may be analyzed for the relative expression level(s) of Neutrokin-alpha, Neutrokin-alphaSV, and/or NAR polynucleotides and/or polypeptides of the invention. The expression level(s) of one or more of these molecules of the invention is (are) then compared to the expression level(s) of the same molecules of the invention as expressed in a person known not to be afflicted with CVID ("the control"). A significant difference in expression level(s) of Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention, and/or NAR polypeptides, and/or agonists and/or antagonists thereof, between samples obtained from the subject and the control suggests that the subject is afflicted with this subset of CVID.

[0553] Cunningham-Rundles and Bodian followed 248 CVID patients over a period of 1-25 years and discovered that a number of associated diseases or conditions appear with increased frequency in CVID patients (Cunningham-Rundles and Bodian, *J. Clin. Immunol.*, 92:34-48 (1999) which is herein incorporated by reference in its entirety.) The most important clinical events include infections, autoimmunity, inflammatory disorders, marked by gastrointestinal and granulomatous disease, cancer and hepatitis. Most CVID patients are at increased risk of recurrent infections particularly of the respiratory tract. The types of acute and recurring bacterial infections exhibited in most patients include pneumonia, bronchitis and sinusitis. Children with CVID have a marked increased risk of otitis media. Additionally, blood borne infections including sepsis, meningitis, septic arthritis, and osteomyelitis are seen with increased frequency in these patients.

[0554] In another specific embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides, or agonists or antagonists thereof (e.g., anti-Neutrokin-alpha, and/or anti-Neutrokin-alphaSV antibodies) are used to diagnose, prognose, treat, or prevent conditions associated with CVID, including, but not limited to, conditions associated with acute and recurring infections (e.g., pneumonia, bronchitis, sinusitis, otitis media, sepsis, meningitis, septic arthritis, and osteomyelitis), chronic lung disease, autoimmunity, granulomatous disease, lymphoma, cancers (e.g., cancers of the breast, stomach, colon, mouth, prostate, lung, vagina, ovary, skin, and melanin forming cells (i.e. melanoma), inflammatory bowel disease (e.g., Crohn's disease, ulcerative colitis, and ulcerative proctitis), malabsorption, Hodgkin's disease, and Waldenstrom's macroglobulinemia.

[0555] In a specific embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides, or agonists or antagonists thereof (e.g., anti-Neutrokin-alpha, and/or anti-Neutrokin-alphaSV antibodies) are used to diagnose, prognose, treat, or prevent a disorder characterized by deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides or polypeptides, or agonists or antagonists thereof (e.g., anti-Neutrokin-alpha and/or anti-Neutrokin-alphaSV antibodies) may be used to diagnose, prognose, treat, or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL, recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carmii.

[0556] In another embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides or Neutrokin-alpha and/or Neutrokin-alphaSV agonists or antagonists (e.g., anti-Neutrokin-alpha and/or anti-Neutrokin-alphaSV antibodies) of the invention are used to treat, diagnose, or prognose an individual having an autoimmune disease or disorder.

[0557] Autoimmune diseases or disorders that may be treated, diagnosed, or prognosed using Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides or Neutrokin-alpha and/or Neutrokin-alphaSV agonists or antagonists (e.g., anti-Neutrokin-alpha and/or anti-Neutrokin-alphaSV antibodies) of the invention include, but are not limited to, one or more of the following: autoimmune hemolytic anemia, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, autoimmune cytopenia, hemolytic anemia, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, glomerulonephritis (e.g., IgA nephropathy), Multiple Sclerosis, Neuritis, Uveitis, Ophthalmia, Polyendocrinopathies, Purpura (e.g., Henoch-Schoenlein purpura), Reiter's Disease, Stiff-Man Syndrome, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye,

autoimmune thyroiditis, hypothyroidism (i.e., Hashimoto's thyroiditis, systemic lupus erythematosus, Goodpasture's syndrome, Pemphigus, Receptor autoimmunities such as, for example, (a) Graves' Disease, (b) Myasthenia Gravis, and (c) insulin resistance, autoimmune hemolytic anemia, autoimmune thrombocytopenic purpura, rheumatoid arthritis, scleroderma with anti-collagen antibodies, mixed connective tissue disease, polymyositis/dermatomyositis, pernicious anemia, idiopathic Addison's disease, infertility, glomerulonephritis such as primary glomerulonephritis and IgA nephropathy, bullous pemphigoid, Sjogren's syndrome, diabetes mellitus, and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis), chronic active hepatitis, primary biliary cirrhosis, other endocrine gland failure, vitiligo, vasculitis, post-MI, cardiotomy syndrome, urticaria, atopic dermatitis, asthma, inflammatory myopathies, and other inflammatory, granulomatous, degenerative, and atrophic disorders.

[0558] According to this embodiment, an individual having an autoimmune disease or disorder expresses aberrantly high levels of Neutrokin-alpha, Neutrokin-alpha SV, and/or NAR when compared to an individual not having an autoimmune disease or disorder. Any means described herein or otherwise known in the art may be applied to detect Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention and/or NAR polypeptides (e.g., FACS analysis or ELISA detection of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention and hybridization or PCR detection of Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides of the invention) and to determine the expression profile of Neutrokin-alpha and/or Neutrokin-alphaSV, polynucleotides and/or polypeptides of the invention and/or NAR polypeptides in a biological sample.

[0559] A biological sample of persons afflicted with an autoimmune disease or disorder is characterized by high levels of expression of Neutrokin-alpha, Neutrokin-alphaSV, and/or NAR when compared to that observed in individuals not having an autoimmune disease or disorder. Thus, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides and/or polypeptides of the invention, and/or agonists or antagonists thereof, may be used according to the methods of the invention in the diagnosis and/or prognosis of an autoimmune disease or disorder. For example, a biological sample obtained from a person suspected of being afflicted with an autoimmune disease or disorder ("the subject") may be analyzed for the relative expression level(s) of Neutrokin-

alpha, and/or Neutrokine-alphaSV polynucleotides and/or polypeptides of the invention and/or NAR polypeptides. The expression level(s) of one or more of these molecules of the invention is (are) then compared to the expression level(s) of the same molecules of the invention as expressed in a person known not to be afflicted with an autoimmune disease or disorder. A significant difference in expression level(s) of Neutrokine-alpha, and/or Neutrokine-alphaSV, polynucleotides and/or polypeptides of the invention, and/or agonists and/or antagonists thereof, and/or NAR polypeptides between samples obtained from the subject and the control suggests that the subject is afflicted with an autoimmune disease or disorder.

[0560] In another embodiment, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides or Neutrokine-alpha and/or Neutrokine-alphaSV agonists or antagonists (e.g., anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies) of the invention are used to treat, diagnose, or prognose an individual having systemic lupus erythematosus or a subset of this disease. According to this embodiment, an individual having systemic lupus erythematosus or a subset of individuals having systemic lupus erythematosus expresses aberrantly high levels of Neutrokine-alpha and/or Neutrokine-alpha SV when compared to an individual not having systemic lupus erythematosus or this subset of systemic lupus erythematosus. Any means described herein or otherwise known in the art may be applied to detect Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention (e.g., FACS analysis or ELISA detection of Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention and hybridization or PCR detection of Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides of the invention) and to determine the expression profile of Neutrokine-alpha and/or Neutrokine-alphaSV, polynucleotides and/or polypeptides of the invention in a biological sample.

[0561] A biological sample of persons afflicted with systemic lupus erythematosus is characterized by high levels of expression of Neutrokine-alpha and/or Neutrokine-alphaSV when compared to that observed in individuals not having systemic lupus erythematosus. Thus, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides and/or polypeptides of the invention, and/or agonists or antagonists thereof, may be used according to the methods of the invention in the diagnosis and/or prognosis of systemic lupus erythematosus or a subset of systemic lupus erythematosus. For example, a

biological sample obtained from a person suspected of being afflicted with systemic lupus erythematosus ("the subject") may be analyzed for the relative expression level(s) of Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides and/or polypeptides of the invention. The expression level(s) of one or more of these molecules of the invention is (are) then compared to the expression level(s) of the same molecules of the invention as expressed in a person known not to be afflicted with systemic lupus erythematosus. A significant difference in expression level(s) of Neutrokin-alpha, and/or Neutrokin-alphaSV, polynucleotides and/or polypeptides of the invention, and/or agonists and/or antagonists thereof, between samples obtained from the subject and the control suggests that the subject is afflicted with systemic lupus erythematosus or a subset thereof.

[0562] Furthermore, there is a direct correlation between the severity of systemic lupus erythematosus, or a subset of this disease, and the concentration of Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides (RNA) and/or polypeptides of the invention. Thus, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides, (RNA), polypeptides and/or agonists or antagonists of the invention, may be used according to the methods of the invention in prognosis of the severity of systemic lupus erythematosus or a subset of systemic lupus erythematosus. For example, a biological sample obtained from a person suspected of being afflicted with systemic lupus erythematosus ("the subject") may be analyzed for the relative expression level(s) of Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides and/or polypeptides of the invention. The expression level(s) of one or more of these molecules of the invention is (are) then compared to the expression level(s) of the same molecules of the invention as expressed in a panel of persons known to represent a range in severities of this disease. According to this method, the match of expression level with a characterized member of the panel indicates the severity of the disease.

[0563] Elevated levels of soluble Neutrokin-alpha have been observed in the serum of patients with Systemic Lupus Erythematosus (SLE). In comparing the sera of 150 SLE patients with that of 38 control individuals, it was found that most of the SLE patients had more than 5ng/ml of serum Neutrokin-alpha, more than 30% of SLE patients had levels greater than 10ng/ml, and approximately 10% of SLE patients had serum Neutrokin-alpha levels greater than 20ng/ml. In contrast, the majority of normal controls had Neutrokin-alpha levels less than 5ng/ml, and less than 10% had levels higher than

10ng/ml. The elevated levels of Neutrokin-alpha protein in sera is present in the soluble form and has biologic activity as assayed by the ability to stimulate anti-IgM treated B cells in vitro. SLE patients with more than 15ng/ml serum Neutrokin-alpha were also found to have elevated levels of anti-dsDNA antibodies compared to both normal controls and SLE patients with less than 5ng/ml of serum Neutrokin-alpha (unpublished data).

[0564] In addition the serum of two subgroups of patients which were positive for anti-nuclear antibodies (ANA+) but did not meet the formal requirements of the American College of Rheumatology (ACR) for classification of SLE were analyzed for Neutrokin-alpha levels. The first subgroup of sera was ANA+ sera that came from patients who did not present with the clinical impression of SLE. This group had only slightly elevated levels of Neutrokin-alpha (~9ng/ml Neutrokin-alpha). The second subgroup however, which was ANA+ sera from patients who presented with the clinical impression of SLE, had significantly increased Neutrokin-alpha levels (~15ng/ml). These results suggest that an elevated level of Neutrokin-alpha precedes the formal fulfillment of the ACR criteria. The ACR criteria are described in Tan, E.M., et al, *Arthritis and Rheumatism* 25:1271 – 1277 (1982).

[0565] In another embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides or Neutrokin-alpha and/or Neutrokin-alphaSV agonists or antagonists (e.g., anti-Neutrokin-alpha and/or anti-Neutrokin-alphaSV antibodies) of the invention are used to treat, diagnose, or prognose an individual having rheumatoid arthritis or a subset of this disease. According to this embodiment, an individual having rheumatoid arthritis or a subset of individuals having rheumatoid arthritis expresses aberrantly high levels of Neutrokin-alpha and/or Neutrokin-alpha SV when compared to an individual not having rheumatoid arthritis or this subset of rheumatoid arthritis. Any means described herein or otherwise known in the art may be applied to detect Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention (e.g., FACS analysis or ELISA detection of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention and hybridization or PCR detection of Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides of the invention) and to determine the expression profile of Neutrokin-alpha and/or Neutrokin-alphaSV, polynucleotides and/or polypeptides of the invention in a biological sample.

[0566] A biological sample of persons afflicted with rheumatoid arthritis is characterized by high levels of expression of Neutrokin-alpha and/or Neutrokin-alphaSV when compared to that observed in individuals not having rheumatoid arthritis. Thus, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides and/or polypeptides of the invention, and/or agonists or antagonists thereof, may be used according to the methods of the invention in the diagnosis and/or prognosis of rheumatoid arthritis or a subset of rheumatoid arthritis. For example, a biological sample obtained from a person suspected of being afflicted with rheumatoid arthritis ("the subject") may be analyzed for the relative expression level(s) of Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides and/or polypeptides of the invention. The expression level(s) of one or more of these molecules of the invention is (are) then compared to the expression level(s) of the same molecules of the invention as expressed in a person known not to be afflicted with rheumatoid arthritis. A significant difference in expression level(s) of Neutrokin-alpha, and/or Neutrokin-alphaSV, polynucleotides and/or polypeptides of the invention, and/or agonists and/or antagonists thereof, between samples obtained from the subject and the control suggests that the subject is afflicted with rheumatoid arthritis or a subset thereof.

[0567] In other specific embodiments, antibodies of the invention which specifically bind to Neutrokin-alpha and/or Neutrokin-alphaSV can be used for diagnostic purposes to detect, diagnose, prognose, or monitor Sjögren's Syndrome or conditions associated therewith. The invention provides for the detection of aberrant expression of Neutrokin-alpha and/or Neutrokin-alphaSV comprising: (a) assaying the expression of Neutrokin-alpha and/or Neutrokin-alphaSV in a biological sample of an individual using one or more antibodies of the invention that immunospecifically binds to Neutrokin-alpha and/or Neutrokin-alphaSV; and (b) comparing the level of Neutrokin-alpha and/or Neutrokin-alphaSV with a standard level of Neutrokin-alpha and/or Neutrokin-alphaSV, *e.g.*, in normal biological samples, whereby an increase in the assayed level of Neutrokin-alpha and/or Neutrokin-alphaSV compared to the standard level of Neutrokin-alpha and/or Neutrokin-alphaSV is indicative of Sjögren's Syndrome.

[0568] In other specific embodiments, antibodies of the invention which specifically bind to Neutrokin-alpha and/or Neutrokin-alphaSV can be used for diagnostic purposes to detect, diagnose, prognose, or monitor HIV infection or conditions associated therewith

(e.g., AIDS) The invention provides for the detection of aberrant expression of Neutrokin-alpha and/or Neutrokin-alphaSV comprising: (a) assaying the expression of Neutrokin-alpha and/or Neutrokin-alphaSV in a biological sample of an individual using one or more antibodies of the invention that immunospecifically binds to Neutrokin-alpha and/or Neutrokin-alphaSV; and (b) comparing the level of Neutrokin-alpha and/or Neutrokin-alphaSV with a standard level of Neutrokin-alpha and/or Neutrokin-alphaSV, *e.g.*, in normal biological samples, whereby an increase in the assayed level of Neutrokin-alpha and/or Neutrokin-alphaSV compared to the standard level of Neutrokin-alpha and/or Neutrokin-alphaSV is indicative of HIV infection.

[0569] In another embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides or Neutrokin-alpha and/or Neutrokin-alphaSV agonists or antagonists (*e.g.*, anti-Neutrokin-alpha and/or anti-Neutrokin-alphaSV antibodies) of the invention are used to treat, diagnose, or prognose an individual with an immune-based rheumatologic diseases, including but not limited to, SLE, rheumatoid arthritis, CREST syndrome (a variant of scleroderma characterized by calcinosis, Raynaud's phenomenon, esophageal motility disorders, sclerodactyly, and telangiectasia.), seronegative spondyloarthropathy (SpA), polymyositis/dermatomyositis, microscopic polyangiitis, hepatitis C-associated arthritis, Takayasu's arteritis, and undifferentiated connective tissue disorder. According to this embodiment, an individual having an immune-based rheumatologic disease or a subset of individuals having a particular immune-based rheumatologic disease expresses aberrantly high levels of Neutrokin-alpha and/or Neutrokin-alpha SV when compared to an individual not having the particular immune-based rheumatologic disease or this subset of individuals having the particular immune-based rheumatologic disease. Any means described herein or otherwise known in the art may be applied to detect Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention (*e.g.*, FACS analysis or ELISA detection of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention and hybridization or PCR detection of Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides of the invention) and to determine the expression profile of Neutrokin-alpha and/or Neutrokin-alphaSV, polynucleotides and/or polypeptides of the invention in a biological sample .

[0570] A biological sample of persons afflicted with an immune-based rheumatologic disease is characterized by high levels of expression of Neutrokin-alpha and/or

Neutrokin-alphaSV when compared to that observed in individuals not having an immune-based rheumatologic disease. Thus, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides and/or polypeptides of the invention, and/or agonists or antagonists thereof, may be used according to the methods of the invention in the diagnosis and/or prognosis of an immune-based rheumatologic disease. For example, a biological sample obtained from a person suspected of being afflicted with an immune-based rheumatologic disease ("the subject") may be analyzed for the relative expression level(s) of Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides and/or polypeptides of the invention. The expression level(s) of one or more of these molecules of the invention is (are) then compared to the expression level(s) of the same molecules of the invention as expressed in a person known not to be afflicted with an immune-based rheumatologic disease. A significant difference in expression level(s) of Neutrokin-alpha, and/or Neutrokin-alphaSV, polynucleotides and/or polypeptides of the invention, and/or agonists and/or antagonists thereof, between samples obtained from the subject and the control suggests that the subject is afflicted with an immune-based rheumatologic disease.

[0571] It has been observed, that serum Neutrokin-alpha levels inversely correlate with nephrotic-range proteinuria (>3gm proteinuria in a 24 hour urine collection) using a sample of 71 SLE patients ($p=0.019$). Proteinuria was determined in 71 SLE patients within one month of phlebotomy for serum Neutrokin-alpha determination. Serum Neutrokin-alpha was classified as low, normal, or high based on the 5th through 95th percentiles for normal controls. Nephrotic-range proteinuria was inversely correlated with serum Neutrokin-alpha levels. Thus, in specific embodiments, serum levels of Neutrokin-alpha in individuals diagnosed with an immune based rheumatologic disease (e.g., SLE, rheumatoid arthritis, CREST syndrome (a variant of scleroderma characterized by calcinosis, Raynaud's phenomenon, esophageal motility disorders, sclerodactyly, and telangiectasia.), seronegative spondyloarthropathy (SpA), polymyositis/dermatomyositis, microscopic polyangiitis, hepatitis C-associated arthritis, Takayasu's arteritis, and undifferentiated connective tissue disorder) may be used to determine, diagnose, prognose, or monitor the severity of certain aspects or symptoms of the disease, such as nephrotic-range proteinuria.

[0572] Thus, the invention provides a diagnostic method useful during diagnosis of a immune system disorder, including cancers of this system, and immunodeficiencies and/or autoimmune diseases which involves measuring the expression level of the gene encoding the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard Neutrokin-alpha and/or Neutrokin-alphaSV gene expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of an immune system disorder.

[0573] Levels of soluble Neutrokin-alpha in the serum of patients with follicular non-Hodgkin's lymphoma are elevated compared to levels of soluble neutrokin-alpha in the sera of healthy individuals. Thus, in a specific embodiment, the invention provides method of diagnosing non-Hodgkin's lymphoma which involves measuring the expression level of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides and/or polynucleotides in immune system tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard Neutrokin-alpha and/or Neutrokin-alphaSV gene expression level, whereby an increase in the gene expression level compared to the standard is indicative of non-Hodgkin's Lymphoma. Other forms of Non-Hodgkin's lymphoma which may be diagnosed according to the above method include, but are not limited to, mantle cell lymphoma, diffuse large cell lymphoma, chronic lymphocytic leukemia, small lymphocytic leukemia, and marginal zone lymphoma.

[0574] Where a diagnosis of a disorder in the immune system, including, but not limited to, diagnosis of a tumor, diagnosis of an immunodeficiency, and/or diagnosis of an autoimmune disease, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed Neutrokin-alpha and/or Neutrokin-alphaSV gene expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

[0575] By analyzing or determining the expression level of the gene encoding the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide is intended qualitatively or quantitatively measuring or estimating the level of the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide or the level of the mRNA encoding the Neutrokin-alpha

and/or Neutrokine-alphaSV polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide level or mRNA level in a second biological sample). Preferably, the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder of the immune system. As will be appreciated in the art, once a standard Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

[0576] By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide or mRNA. As indicated, biological samples include body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) which contain free extracellular domains of the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide, immune system tissue, and other tissue sources found to express complete or free extracellular domain of the Neutrokine-alpha and/or Neutrokine-alphaSV or a Neutrokine-alpha and/or Neutrokine-alphaSV receptor. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

[0577] The compounds of the present invention are useful for diagnosis, prognosis, or treatment of various immune system-related disorders in mammals, preferably humans. Such disorders include, but are not limited to tumors (e.g., B cell and monocytic cell leukemias and lymphomas, See Example) and tumor metastasis, infections by bacteria, viruses and other parasites, immunodeficiencies, inflammatory diseases, lymphadenopathy, autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, Sjogren syndrome, mixed connective tissue disease, and inflammatory myopathies), and graft versus host disease.

[0578] Total cellular RNA can be isolated from a biological sample using any suitable technique such as the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, *Anal. Biochem.* 162:156-159 (1987). Levels of mRNA encoding the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide are then assayed using any appropriate method. These include Northern blot analysis, S1 nuclease mapping, the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

[0579] Assaying Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide levels in a biological sample can occur using antibody-based techniques. For example, Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., *et al.*, *J. Cell. Biol.* 101:976-985 (1985); Jalkanen, M., *et al.*, *J. Cell. Biol.* 105:3087-3096 (1987)). Other antibody-based methods useful for detecting Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). Suitable antibody assay labels are known in the art and include enzyme labels, (e.g., glucose oxidase, alkaline phosphatase and horse radish peroxidase) and radioisotopes, such as iodine (^{131}I , ^{125}I , ^{123}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115m}In , ^{113m}In , ^{112}In , ^{111}In), and technetium (^{99}Tc , ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , ^{97}Ru ; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0580] Techniques known in the art may be applied to label antibodies of the invention. Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety) and direct coupling reactions (e.g., Bolton-Hunter, Chloramine-T reaction, and Iodogen®-based labelling).

[0581] The tissue or cell type to be analyzed will generally include those which are known, or suspected, to express the Neutrokin-alpha gene (such as, for example, cells of monocytic lineage) or cells or tissue which are known, or suspected, to express the Neutrokin-alpha receptor gene (such as, for example, cells of B cell lineage and the spleen). The protein isolation methods employed herein may, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), which is incorporated herein by reference in its entirety. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step in the assessment of cells that could be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the Neutrokin-alpha gene or Neutrokin-alpha receptor gene.

[0582] For example, antibodies, or fragments of antibodies, such as those described herein, may be used to quantitatively or qualitatively detect the presence of Neutrokin-alpha gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection.

[0583] The antibodies (or fragments thereof) or Neutrokin-alpha polypeptides or polypeptides of the present invention may, additionally, be employed histologically, as in immunofluorescence, immunoelectron microscopy or non-immunological assays, for in situ detection of Neutrokin-alpha gene products or conserved variants or peptide fragments thereof, or for Neutrokin-alpha binding to Neutrokin-alpha receptor. In situ detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody or Neutrokin-alpha polypeptide of the present invention. The antibody (or fragment) or Neutrokin-alpha polypeptide is preferably applied by overlaying the labeled antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the Neutrokin-alpha gene product, or conserved variants or peptide fragments, or Neutrokin-alpha polypeptide binding, but also its distribution in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide

variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

[0584] Immunoassays and non-immunoassays for Neutrokin-alpha gene products or conserved variants or peptide fragments thereof will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells which have been incubated in cell culture, in the presence of a detectably labeled antibody capable of identifying Neutrokin-alpha gene products or conserved variants or peptide fragments thereof, and detecting the bound antibody by any of a number of techniques well-known in the art.

[0585] Immunoassays and non-immunoassays for Neutrokin-alpha receptor gene products or conserved variants or peptide fragments thereof will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells which have been incubated in cell culture, in the presence of a detectable or labeled Neutrokin-alpha polypeptide capable of identifying Neutrokin-alpha receptor gene products or conserved variants or peptide fragments thereof, and detecting the bound Neutrokin-alpha polypeptide by any of a number of techniques well-known in the art.

[0586] The biological sample may be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled anti-Neutrokin-alpha antibody or detectable Neutrokin-alpha polypeptide. The solid phase support may then be washed with the buffer a second time to remove unbound antibody or polypeptide. Optionally the antibody is subsequently labeled. The amount of bound label on solid support may then be detected by conventional means.

[0587] By "solid phase support or carrier" is intended any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external

surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

[0588] The binding activity of a given lot of anti-Neutrokin-alpha antibody or Neutrokin-alpha polypeptide may be determined according to well-known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

[0589] In addition to assaying Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide levels or polynucleotide levels in a biological sample obtained from an individual, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or polynucleotides can also be detected *in vivo* by imaging. For example, in one embodiment of the invention, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide and/or anti-Neutrokin-alpha antibody is used to image B cell lymphomas. In another embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides and/or anti-Neutrokin-alpha antibodies and/or Neutrokin-alpha polynucleotides of the invention (e.g., polynucleotides complementary to all or a portion of Neutrokin-alpha and/or Neutrokin-alphaSV mRNA) is used to image lymphomas (e.g., monocyte and B cell lymphomas) .

[0590] Antibody labels or markers for *in vivo* imaging of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide include those detectable by X-radiography, NMR, MRI, CAT-scans or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma. Where *in vivo* imaging is used to detect enhanced levels of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide for diagnosis in humans, it may be preferable to use human antibodies or "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using techniques described herein or otherwise known in the art. For example methods for producing chimeric antibodies are known in the art. See, for review, Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et

al., WO 8702671; Boulianne *et al.*, *Nature* 312:643 (1984); Neuberger *et al.*, *Nature* 314:268 (1985).

[0591] Additionally, any Neutrokin-alpha polypeptide whose presence can be detected, can be administered. For example, Neutrokin-alpha polypeptides labeled with a radio-opaque or other appropriate compound can be administered and visualized *in vivo*, as discussed, above for labeled antibodies. Further such Neutrokin-alpha polypeptides can be utilized for *in vitro* diagnostic procedures.

[0592] A Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, iodine (^{131}I , ^{125}I , ^{123}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium ($^{115\text{m}}\text{In}$, $^{113\text{m}}\text{In}$, ^{112}In , ^{111}In), and technetium (^{99}Tc , $^{99\text{m}}\text{Tc}$), thallium (^{201}Ti), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , ^{97}Ru), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of $^{99\text{m}}\text{Tc}$. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain Neutrokin-alpha protein. *In vivo* tumor imaging is described in S.W. Burchiel *et al.*, "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

[0593] With respect to antibodies, one of the ways in which the anti-Neutrokin-alpha antibody can be detectably labeled is by linking the same to an enzyme and using the linked product in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller *et al.*, *J. Clin. Pathol.* 31:507-520 (1978); Butler, J.E., *Meth. Enzymol.* 73:482-523 (1981); Maggio, E. (ed.), 1980, Enzyme Immunoassay, CRC Press, Boca Raton, FL; Ishikawa, E. *et al.*, (eds.),

1981, Enzyme Immunoassay, Kgaku Shoin, Tokyo). The enzyme which is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. Additionally, the detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

[0594] Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the antibodies or antibody fragments, it is possible to detect Neutrokinne-alpha through the use of a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by means including, but not limited to, a gamma counter, a scintillation counter, or autoradiography.

[0595] It is also possible to label the antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave-length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, ophthaldehyde and fluorescamine.

[0596] The antibody can also be detectably labeled using fluorescence emitting metals such as ¹⁵²Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

[0597] The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is

then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0598] Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling include, but are not limited to, luciferin, luciferase and aequorin.

Treatment of Immune System-Related Disorders

[0599] As noted above, Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides and polypeptides, and anti-Neutrokine-alpha antibodies, are useful for diagnosis of conditions involving abnormally high or low expression of Neutrokine-alpha and/or Neutrokine-alphaSV activities. Given the cells and tissues where Neutrokine-alpha and/or Neutrokine-alphaSV is expressed as well as the activities modulated by Neutrokine-alpha and/or Neutrokine-alphaSV, it is readily apparent that a substantially altered (increased or decreased) level of expression of Neutrokine-alpha and/or Neutrokine-alphaSV in an individual compared to the standard or "normal" level produces pathological conditions related to the bodily system(s) in which Neutrokine-alpha and/or Neutrokine-alphaSV is expressed and/or is active.

[0600] It will also be appreciated by one of ordinary skill that, since the Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides of the invention are members of the TNF family, the extracellular domains of the respective proteins may be released in soluble form from the cells which express Neutrokine-alpha and/or Neutrokine-alphaSV by proteolytic cleavage and therefore, when Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide (particularly a soluble form of the respective extracellular domains) is added from an exogenous source to cells, tissues or the body of an individual, the polypeptide will exert its modulating activities on any of its target cells of that individual. Also, cells expressing this type II transmembrane protein may be added to cells, tissues or the body of an individual whereby the added cells will bind to cells expressing receptor for

Neutrokine-alpha and/or Neutrokine-alphaSV whereby the cells expressing Neutrokine-alpha and/or Neutrokine-alphaSV can cause responses (e.g., proliferation or cytotoxicity) in the receptor-bearing target cells.

[0601] In one embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells, such as, for example, B cells expressing Neutrokine-alpha and/or Neutrokine-alphaSV receptor, or monocytes expressing the cell surface bound form of Neutrokine-alpha and/or Neutrokine-alphaSV. Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

[0602] In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (e.g., Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides or anti-Neutrokine-alpha and/or anti-Neutrokine-alphaSV antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

[0603] In another embodiment, the invention provides for a method of killing cells of hematopoietic origin, comprising, or alternatively consisting of, contacting Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides with cells of hematopoietic origin. In specific embodiments, the method of killing cells of hematopoietic origin, comprises, or alternatively consists of, administering to an animal in which such killing is desired, a Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide in an amount effective to kill cells of hematopoietic origin. Cells of hematopoietic origin include, but are not limited to, lymphocytes (e.g., B cells and T cells), monocytes, macrophages, dendritic cells, polymorphonuclear leukocytes (e.g., basophils, eosinophils, neutrophils), mast cells,

platelets, erythrocytes and progenitor cells of these lineages. Cells of hematopoietic origin include, but are not limited to, healthy and diseased cell as found present in an animal, preferably a mammal and most preferably a human, or as isolated from an animal, transformed cells, cell lines derived from the above listed cell types, and cell cultures derived from the above listed cell types. Cells of hematopoietic origin may be found or isolated in, for example, resting, activated or anergic states.

[0604] In another embodiment, the invention provides a method for the specific destruction (i.e., killing) of cells (e.g., the destruction of tumor cells) by administering Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide conjugates of the invention (e.g., Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides conjugated with radioisotopes, toxins, or cytotoxic prodrugs) in which such destruction of cells is desired.

[0605] In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or anti-Neutrokin-alpha and/or anti-Neutrokin-alphaSV antibodies) in association with toxins or cytotoxic prodrugs.

[0606] In a specific embodiment, the invention provides a method for the specific destruction of cells of B cell lineage (e.g., B cell related leukemias or lymphomas) by administering Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides in association with toxins or cytotoxic prodrugs.

[0607] In another specific embodiment, the invention provides a method for the specific destruction of cells of monocytic lineage (e.g., monocytic leukemias or lymphomas) by administering anti-Neutrokin-alpha and/or anti-Neutrokin-alphaSV antibodies in association with toxins or cytotoxic prodrugs.

[0608] Biodistribution studies (See Example 12) of radiolabeled Neutrokin-alpha polypeptide (amino acids 134-285 of SEQ ID NO:2) that had been injected into BALB/c mice demonstrated that Neutrokin-alpha has high in vivo targeting specificity for lymphoid tissues such as spleen and lymph nodes. Thus in specific embodiments, the invention provides a method for the specific destruction or disablement of lymphoid tissue (e.g., lymph nodes and spleen) by administering Neutrokin-alpha, Neutrokin-alphaSV, anti-Neutrokin-alpha, and/or anti-Neutrokin-alphaSV polypeptides in association with

radioisotopes, toxins or cytotoxic prodrugs. In preferred embodiments, the lymphoid tissue is not permanently destroyed, but rather is temporarily disabled, (e.g., cells of hematopoietic lineage in lymphoid tissues are destroyed/killed while Neutrokine-alpha, Neutrokine-alphaSV, anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are administered, but these populations recover once administration of Neutrokine-alpha, Neutrokine-alphaSV, anti-Neutrokine-alpha, and/or anti-Neutrokine-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs is stopped.)

[0609] By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, cytotoxins (cytotoxic agents), or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ²¹³Bi, or other radioisotopes such as, for example, ¹⁰³Pd, ¹³³Xe, ¹³¹I, ⁶⁸Ge, ⁵⁷Co, ⁶⁵Zn, ⁸⁵Sr, ³²P, ³⁵S, ⁹⁰Y, ¹⁵³Sm, ¹⁵³Gd, ¹⁶⁹Yb, ⁵¹Cr, ⁵⁴Mn, ⁷⁵Se, ¹¹³Sn, ⁹⁰Yttrium, ¹¹⁷Tin, ¹⁸⁶Rhenium, ¹⁶⁶Holmium, and ¹⁸⁸Rhenium; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[0610] Techniques known in the art may be applied to label polypeptides and antibodies of the invention. Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety) and direct coupling reactions (e.g., Bolton-Hunter, Chloramine-T reaction, and Iodogen® based labeling methods).

[0611] A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine,

mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa, chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

[0612] By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

[0613] In specific embodiments, Neutrokin-alpha, Neutrokin-alphaSV, anti-Neutrokin-alpha, and/or anti-Neutrokin-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of autoimmune diseases. In preferred emodiments, Neutrokin-alpha, Neutrokin-alphaSV, anti-Neutrokin-alpha, and/or anti-Neutrokin-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of systemic lupus erythematosus. Neutrokin-alpha, Neutrokin-alphaSV, anti-Neutrokin-alpha, and/or anti-Neutrokin-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of rheumatoid arthritis including advanced rheumatoid arthritis. In preferred emodiments, Neutrokin-alpha, Neutrokin-alphaSV, anti-Neutrokin-alpha, and/or anti-Neutrokin-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of idiopathic thrombocytopenic purpura (ITP).

[0614] In other preferred embodiments Neutrokin-alpha, Neutrokin-alphaSV, anti-Neutrokin-alpha, and/or anti-Neutrokin-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of Sjögren's syndrome. In other preferred embodiments, Neutrokin-alpha, Neutrokin-alphaSV, anti-Neutrokin-alpha, and/or anti-Neutrokin-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of IgA nephropathy. In other preferred embodiments, Neutrokin-alpha, Neutrokin-alphaSV, anti-Neutrokin-alpha, and/or anti-Neutrokin-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of Myasthenia gravis. In preferred embodiments, Neutrokin-alpha, Neutrokin-alphaSV, anti-Neutrokin-alpha, and/or anti-Neutrokin-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of multiple sclerosis. In still other preferred embodiments, Neutrokin-alpha, Neutrokin-alphaSV, anti-Neutrokin-alpha, and/or anti-Neutrokin-alphaSV polypeptides in association with radioisotopes, toxins or cytotoxic prodrugs are used to treat or ameliorate the symptoms of vasculitis.

[0615] In one embodiment, the invention provides methods and compositions for inhibiting or reducing immunoglobulin production (e.g. IgM, IgG, and/or IgA production), comprising, or alternatively consisting of, contacting an effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide inhibits or reduces Neutrokin-alpha and/or Neutrokin-alphaSV mediated immunoglobulin production. In specific embodiments, the invention provides methods and compositions for inhibiting or reducing immunoglobulin production (e.g. IgM, IgG, and/or IgA production) in response to T cell dependent antigens, comprising, or alternatively consisting of, contacting an effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide inhibits or reduces Neutrokin-alpha and/or Neutrokin-alphaSV mediated immunoglobulin production in response to T cell dependent antigens. In specific embodiments, the invention provides methods and compositions for inhibiting or reducing immunoglobulin production (e.g. IgM, IgG, and/or

IgA production) in response to T cell independent antigens, comprising, or alternatively consisting of, contacting an effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide inhibits or reduces Neutrokin-alpha and/or Neutrokin-alphaSV mediated immunoglobulin production in response to T cell independent antigens.

[0616] In another embodiment, the invention provides methods and compositions for inhibiting or reducing immunoglobulin production (e.g. IgM, IgG, and/or IgA production), comprising, or alternatively consisting of, administering to an animal in which such inhibition or reduction is desired, a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in an amount effective to inhibit or reduce immunoglobulin production. In another embodiment, the invention provides methods and compositions for inhibiting or reducing immunoglobulin production (e.g. IgM, IgG, and/or IgA production) in response to T cell dependent antigens, comprising, or alternatively consisting of, administering to an animal in which such inhibition or reduction is desired, a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in an amount effective to inhibit or reduce immunoglobulin production in response to T cell dependent antigens. In another embodiment, the invention provides methods and compositions for inhibiting or reducing immunoglobulin production (e.g. IgM, IgG, and/or IgA production) in response to T cell independent antigens, comprising, or alternatively consisting of, administering to an animal in which such inhibition or reduction is desired, a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in an amount effective to inhibit or reduce immunoglobulin production in response to T cell independent antigens.

[0617] In another embodiment, the invention provides methods and compositions for stimulating immunoglobulin production (e.g. IgM, IgG, and/or IgA production), comprising, or alternatively consisting of, contacting an effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide stimulates Neutrokin-alpha and/or Neutrokin-alphaSV mediated immunoglobulin production. In another embodiment, the invention provides methods and compositions for stimulating immunoglobulin production (e.g. IgM, IgG, and/or IgA production) in response to T cell dependent antigens comprising, or alternatively consisting of,

contacting an effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide stimulates Neutrokin-alpha and/or Neutrokin-alphaSV mediated immunoglobulin production in response to T cell dependent antigens. In another embodiment, the invention provides methods and compositions for stimulating immunoglobulin production (e.g. IgM, IgG, and/or IgA production) in response to T cell independent antigens comprising, or alternatively consisting of, contacting an effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide stimulates Neutrokin-alpha and/or Neutrokin-alphaSV mediated immunoglobulin production in response to T cell independent antigens.

[0618] In another embodiment, the invention provides methods and compositions for stimulating immunoglobulin production (e.g. IgM, IgG, and/or IgA production) comprising, or alternatively consisting of, administering to an animal in which such stimulation is desired, a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in an amount effective to stimulate immunoglobulin production. In another embodiment, the invention provides methods and compositions for stimulating immunoglobulin production (e.g. IgM, IgG, and/or IgA production) in response to T cell dependent antigens comprising, or alternatively consisting of, administering to an animal in which such stimulation is desired, a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in an amount effective to stimulate immunoglobulin production in response to T cell dependent antigens.

[0619] In another embodiment, the invention provides methods and compositions for stimulating immunoglobulin production (e.g. IgM, IgG, and/or IgA production) in response to T cell independent antigens comprising, or alternatively consisting of, administering to an animal in which such stimulation is desired, a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in an amount effective to stimulate immunoglobulin production in response to T cell independent antigens.

[0620] Determination of immunoglobulin levels are most often performed by comparing the level of immunoglobulin in a sample to a standard containing a known amount of immunoglobulin using ELISA assays. Determination of immunoglobulin

levels in a given sample, can readily be determined using ELISA or other method known in the art.

[0621] Receptors belonging to the TNF receptor (TNFR) super family (e.g., TACI and BCMA, receptors to which Neutrokinne-alpha polypeptides bind) can be classified into two types based on the presence or absence of a conserved cytoplasmic domain responsible for apoptosis called a "death domain." TNF receptors without death domains, such as TNF-R2 HVEM/ATAR, RANK, CD27, CD30, CD40, and OX40 interact with TNF receptor associated factors (TRAF 1-6) and mediate anti-apoptotic survival and or proliferative responses via activation of the transcription factor NF-kappaB (reviewed in Wajant et al., Cytokine and Growth Factor Reviews 10(1):15-26, 1999). TACI and BCMA do not contain death domains.

[0622] Investigation of Neutrokinne-alpha (which bind TACI and BCMA) induced signaling in human tonsillar B cells co-stimulated with Staph. Aureus Cowan consistently revealed that mRNA for ERK-1 and PLK were upregulated by Neutrokinne-alpha + SAC treatment (see Example 11). Polo like kinases (PLK) belong to a sub family of serine/threonine kinases related to *Saccharomyces cerevisiae* cell cycle protein CDC5 (29). The expression of PLK is induced during G2 and S phase of the cell cycle. PLK is reported to play a role in cell proliferation (Lee et al., Proc. Natl. Acad. Sci. 95:9301 – 9306). The role or extracellular-signal related kinases (ERK1/2) in cell survival and proliferative effects of growth factors and other agonists has been extensively studied. The induced expression of PLK and ERK-1 is consistent with the survival and proliferative effects of Neutrokinne-alpha on B cells.

[0623] Additionally, in some samples of human tonsillar B cells stimulated with Neutrokinne-alpha and SAC, mRNA for CD25 (IL-2Ralpha) was upregulated. Nuclear extracts from Human tonsillar B cells treated with Neutrokinne-alpha and from IM-9 cells treated with Neutrokinne-alpha were able to shift probes from the CD25 promoter region containing sites for NF-kappaB, SRF, ELF-1 and HMG1/Y in an electromobility shift assay. ELF-1 for example, is a transcription factor that is part of the ETS family of proteins and whose expression appears to be restricted to T and B cells. Binding sites for ELF-1 have been described in the promoters of a number of proteins that are important in the regulation of the immune response.

[0624] Thus, Neutrokin-alpha induced signaling has been shown to be consistent with the activation of cellular activation and cellular proliferation pathways as well as with cellular signaling pathways that regulate B cell lifespan. Further, Neutrokin-alpha and/or Neutrokin-alphaSV treatment of B cells induces cellular proliferation immunoglobulin secretion, a characteristic of activated B cells (Moore et al., Science 285:260-263, 1999). Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides may inhibit, stimulate, or not significantly alter these Neutrokin-alpha and/or Neutrokin-alphaSV mediated activities.

[0625] In one embodiment, the invention provides methods and compositions for inhibiting or reducing proliferation of cells of hematopoietic origin, comprising, or alternatively consisting of, contacting an effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide inhibits or reduces Neutrokin-alpha and/or Neutrokin-alphaSV mediated proliferation of cells of hematopoietic origin. In another embodiment, the invention provides methods and compositions for inhibiting or reducing proliferation of cells of hematopoietic origin comprising, or alternatively consisting of, administering to an animal in which such inhibition or reduction is desired, a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in an amount effective to inhibit or reduce B cell proliferation. In preferred embodiments, the cells of hematopoietic origin are B cells.

[0626] In one embodiment, the invention provides methods and compositions for stimulating proliferation of cells of hematopoietic origin, comprising, or alternatively consisting of, contacting an effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide stimulates Neutrokin-alpha and/or Neutrokin-alphaSV mediated proliferation of cells of hematopoietic origin. In another embodiment, the invention provides methods and compositions for stimulating proliferation of cells of hematopoietic origin comprising, or alternatively consisting of, administering to an animal in which such stimulation is desired, a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in an amount effective to stimulate B cell proliferation. In preferred embodiments, the cells of hematopoietic origin are B cells. B cell proliferation is most commonly assayed in the art by measuring tritiated thymidine incorporation (see Examples 6 & 7). This and other assays are commonly known in the

art and could be routinely adapted for the use of determining the effect of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides on B cell proliferation.

[0627] In one embodiment, the invention provides methods and compositions for inhibiting or reducing activation of cells of hematopoietic origin, comprising, or alternatively consisting of, contacting an effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide inhibits or reduces Neutrokin-alpha and/or Neutrokin-alphaSV mediated activation of cells of hematopoietic origin. In one embodiment, the invention provides methods and compositions for inhibiting or reducing activation of cells of hematopoietic origin, comprising, or alternatively consisting of, administering to an animal in which such inhibition or reduction is desired, a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in an amount effective to inhibit or reduce activation of cells of hematopoietic origin. In preferred embodiments, the cells of hematopoietic origin are B cells.

[0628] In one embodiment, the invention provides methods and compositions for increasing activation of cells of hematopoietic origin, comprising, or alternatively consisting of, contacting an effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide increases Neutrokin-alpha and/or Neutrokin-alphaSV mediated activation of cells of hematopoietic origin. In one embodiment, the invention provides methods and compositions for increasing activation of cells of hematopoietic origin, comprising, or alternatively consisting of, administering to an animal in which such increase is desired, a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in an amount effective to increase activation of cells of hematopoietic origin. In preferred embodiments, the cells of hematopoietic origin are B cells.

[0629] B cell activation can measured in a variety of ways, such as FACS analysis of activation markers expressed on B cells. B cells activation markers include, but are not limited to, CD26, CD 28, CD 30, CD 38, CD 39, CD 69, CD70 CD71 , CD 77, CD 83, CD126, CDw130, and B220. Additionally, B cell activation may be measured by analysis of the activation of signaling molecules involved in B cell activation. By way of non-limiting example, such analysis may take the form of analyzing mRNA levels of signaling

molecules by Northern analysis or real time PCR (See Example 11). One can also measure, for example, the phosphorylation of signaling molecules using anti-phosphotyrosine antibodies in a Western blot. B cell activation may also be measured by measuring the calcium levels in B cells. These and other methods of determining B cell activation are commonly known in the art and could be routinely adapted for the use of determining the effect of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides on B cell activation.

[0630] In one embodiment, the invention provides methods and compositions for decreasing lifespan of cells of hematopoietic origin, comprising, or alternatively consisting of, contacting an effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide inhibits or reduces Neutrokin-alpha and/or Neutrokin-alphaSV regulated lifespan of cells of hematopoietic origin. In one embodiment, the invention provides methods and compositions for decreasing lifespan of cells of hematopoietic origin, comprising, or alternatively consisting of, administering to an animal in which such decrease is desired, a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in an amount effective to decrease lifespan of cells of hematopoietic origin. In preferred embodiments, the cells of hematopoietic origin are B cells.

[0631] In one embodiment, the invention provides methods and compositions for increasing lifespan of cells of hematopoietic origin, comprising, or alternatively consisting of, contacting an effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide with cells of hematopoietic origin, wherein the effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide increases Neutrokin-alpha and/or Neutrokin-alphaSV regulated lifespan of cells of hematopoietic origin. In one embodiment, the invention provides methods and compositions for increasing lifespan of cells of hematopoietic origin, comprising, or alternatively consisting of, administering to an animal in which such increase is desired, a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide in an amount effective to increase lifespan of cells of hematopoietic origin. In preferred embodiments, the cells of hematopoietic origin are B cells.

[0632] B cell life span in vivo may be measured by 5-bromo-2'-deoxyuridine (BrdU) labeling experiments which are well known to one skilled in the art. BrdU is a thymidine

analogue that gets incorporated into the DNA of dividing cells. Cells containing BrdU in their DNA can be detected using, for example fluorescently labeled anti-BrdU antibody and flow cytometry. Briefly, an animal is injected with BrdU in an amount sufficient to label developing B cells. Then, a sample of B cells is withdrawn from the animal, for example, from peripheral blood, and analyzed for the percentage of cells that contain BrdU. Such an analysis performed at several time points can be used to calculate the half life of B cells. Alternatively, B cell survival may be measured in vitro. For example B cells may be cultured under conditions where proliferation does not occur, (for example the media should contain no reagents that crosslink the immunoglobulin receptor, such as anti-IgM antibodies) for a period of time (usually 2-4 days). At the end of this time, the percent of surviving cells is determined, using for instance, the vital dye Trypan Blue, or by staining cells with propidium iodide or any other agent designed to specifically stain apoptotic cells and analyzing the percentage of cells stained using flow cytometry. One could perform this experiment under several conditions, such as B cells treated with Neutrokin-alpha, B cells treated with Neutrokin-alpha and/or Neutrokin-alphaSV-polypeptide complexes, and untreated B cells in order to determine the effects of Neutrokin-alpha and/or Neutrokin-alphaSV and Neutrokin-alpha polypeptides on B cells survival. These and other methods for determining B cell lifespan are commonly known in the art and could routinely be adapted to determining the effect of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides on Neutrokin-alpha and/or Neutrokin-alphaSV regulated B cell lifespan.

[0633] It will be appreciated that conditions caused by a decrease in the standard or normal level of Neutrokin-alpha and/or Neutrokin-alphaSV activity in an individual, particularly disorders of the immune system, can be treated by administration of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide (in the form of soluble extracellular domain or cells expressing the complete protein) or agonist. Thus, the invention also provides a method of treatment of an individual in need of an increased level of Neutrokin-alpha and/or Neutrokin-alphaSV activity comprising administering to such an individual a pharmaceutical composition comprising an amount of an isolated Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide of the invention, or agonist thereof, effective to increase the Neutrokin-alpha and/or Neutrokin-alphaSV activity level in such an individual.

[0634] It will also be appreciated that conditions caused by a increase in the standard or normal level of Neutrokin-alpha and/or Neutrokin-alphaSV activity in an individual, particularly disorders of the immune system, can be treated by administration of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides (in the form of soluble extracellular domain or cells expressing the complete protein) or antagonist (e.g., an anti-Neutrokin-alpha antibody). Thus, the invention also provides a method of treatment of an individual in need of an decreased level of Neutrokin-alpha and/or Neutrokin-alphaSV activity comprising administering to such an individual a pharmaceutical composition comprising an amount of an isolated Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide of the invention, or antagonist thereof, effective to decrease the Neutrokin-alpha and/or Neutrokin-alphaSV activity level in such an individual. A non-limiting example of a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide of the invention that can be administered to an individual in need of an decreased level of Neutrokin-alpha and/or Neutrokin-alphaSV activity, is a dominant negative mutant of a Neutrokin-alpha and/or Neutrokin-alphaSV, which binds to a Neutrokin-alpha and/or Neutrokin-alphaSV receptor but that does not induce signal transduction.

[0635] Autoantibody production is common to several autoimmune diseases and contributes to tissue destruction and exacerbation of disease. Autoantibodies can also lead to the occurrence of immune complex deposition complications and lead to many symptoms of systemic lupus erythromatosis, including kidney failure, neuralgic symptoms and death. Modulating antibody production independent of cellular response would also be beneficial in many disease states. B cells have also been shown to play a role in the secretion of arthritogenic immunoglobulins in rheumatoid arthritis, (Korganow et al., Immunity 10:451-61, 1999) . As such, inhibition of Neutrokin alpha-mediated antibody production would be beneficial in treatment of autoimmune diseases such as myasthenia gravis and rheumatoid arthritis. Compounds of the invention that selectively block or neutralize the action of B-lymphocytes would be useful for such purposes. To verify these capabilities in compositions of the present invention, such compositions are evaluated using assays known in the art and described herein.

[0636] The invention provides methods employing compositions of the invention (e.g., Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the

invention and/or agonists and/or antagonists thereof) for selectively blocking or neutralizing the actions of B-cells in association with end stage renal diseases, which may or may not be associated with autoimmune diseases. Such methods would also be useful for treating immunologic renal diseases. Such methods would be useful for treating glomerulonephritis associated with diseases such as membranous nephropathy, IgA nephropathy or Berger's Disease, IgM nephropathy, Goodpasture's Disease, post-infectious glomerulonephritis, mesangioproliferative disease, minimal-change nephrotic syndrome. Such methods would also serve as therapeutic applications for treating secondary glomerulonephritis or vasculitis associated with such diseases as lupus, polyarteritis, Henoch-Schonlein, Scleroderma, HIV-related diseases, amyloidosis or hemolytic uremic syndrome. The methods of the present invention would also be useful as part of a therapeutic application for treating interstitial nephritis or pyelonephritis associated with chronic pyelonephritis, analgesic abuse, nephrocalcinosis, nephropathy caused by other agents, nephrolithiasis, or chronic or acute interstitial nephritis.

[0637] The methods of the present invention also include use of compositions of the invention in the treatment of hypertensive or large vessel diseases, including renal artery stenosis or occlusion and cholesterol emboli or renal emboli.

[0638] The present invention also provides methods for diagnosis and treatment of renal or urological neoplasms, multiple myelomas, lymphomas, light chain neuropathy or amyloidosis.

[0639] The invention also provides methods for blocking or inhibiting activated B cells using compositions of the invention for the treatment of asthma and other chronic airway diseases such as bronchitis and emphysema.

[0640] Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention, or agonists of Neutrokin-alpha, and/or Neutrokin-alphaSV, can be used in the treatment of infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides or polypeptides, or agonists of Neutrokin-alpha, and/or Neutrokin-alphaSV, may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

[0641] Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated by Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides or polypeptides, or agonists or antagonists of Neutrokine-alpha, and/or Neutrokine-alphaSV. Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. Neutrokine-alpha, and/or Neutrokine-alphaSV polynucleotides or polypeptides, or agonists or antagonists of Neutrokine-alpha, and/or Neutrokine-alphaSV, can be used to treat, prevent, diagnose, and/or detect any of these symptoms or diseases. In specific embodiments, Neutrokine alpha polynucleotides, polypeptides, or agonists are used to treat, prevent, and/or diagnose: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment Neutrokine alpha polynucleotides, polypeptides, or agonists are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment, Neutrokine alpha polynucleotides, polypeptides, or agonists are used to treat, prevent, and/or diagnose AIDS. In an additional specific embodiment Neutrokine-alpha and/or Neutrokine-alphaSV and/or Neutrokine-alpha Receptor polynucleotides, polypeptides, agonists, and/or antagonists are used to treat, prevent, and/or diagnose patients with cryptosporidiosis.

[0642] Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated by Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides or polypeptides, or agonists or antagonists of Neutrokin-alpha, and/or Neutrokin-alphaSV, include, but not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., *Corynebacterium*, *Mycobacterium*, *Nocardia*), *Cryptococcus neoformans*, Aspergillosis, *Bacillaceae* (e.g., *Anthrax*, *Clostridium*), *Bacteroidaceae*, Blastomycosis, *Bordetella*, *Borrelia* (e.g., *Borrelia burgdorferi*, Brucellosis, Candidiasis, *Campylobacter*, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, *E. coli* (e.g., Enterotoxigenic *E. coli* and Enterohemorrhagic *E. coli*), Enterobacteriaceae (*Klebsiella*, *Salmonella* (e.g., *Salmonella typhi*, and *Salmonella paratyphi*), *Serratia*, *Yersinia*), *Erysipelothrix*, *Helicobacter*, Legionellosis, Leptospirosis, *Listeria* (e.g., *Listeria monocytogenes*), Mycoplasmatales, *Mycobacterium leprae*, *Vibrio cholerae*, *Neisseriaceae* (e.g., *Acinetobacter*, Gonorrhea, Menigococcal), *Meisseria meningitidis*, Pasteurellacea Infections (e.g., *Actinobacillus*, *Heamophilus* (e.g., *Heamophilus influenza* type B), *Pasteurella*), *Pseudomonas*, Rickettsiaceae, Chlamydiaceae, Syphilis, *Shigella* spp., Staphylococcal, Meningiococcal, Pneumococcal and Streptococcal (e.g., *Streptococcus pneumoniae* and Group B Streptococcus). These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., meningitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides or polypeptides, or agonists or antagonists of Neutrokin-alpha, and/or Neutrokin-alphaSV, can be used to treat, prevent, diagnose, and/or detect any of these symptoms or diseases. In specific embodiments, Neutrokin alpha polynucleotides, polypeptides, or agonists thereof are

used to treat, prevent, and/or diagnose: tetanus, Diphteria, botulism, and/or meningitis type B.

[0643] Moreover, parasitic agents causing disease or symptoms that can be treated by Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides or polypeptides, or agonists of Neutrokin-alpha, and/or Neutrokin-alphaSV, include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., *Plasmodium virax*, *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale*). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides or polypeptides, or agonists or antagonists of Neutrokin-alpha, and/or Neutrokin-alphaSV, can be used to treat, prevent, diagnose, and/or detect any of these symptoms or diseases. In specific embodiments, Neutrokin alpha polynucleotides, polypeptides, or agonists thereof are used to treat, prevent, and/or diagnose malaria.

[0644] In another embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, are used to treat, prevent, and/or diagnose inner ear infection (such as, for example, otitis media), as well as other infections characterized by infection with *Streptococcus pneumoniae* and other pathogenic organisms.

[0645] In a specific embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides, or agonists or antagonists thereof (e.g., anti-Neutrokin-alpha, and/or anti-Neutrokin-alphaSV antibodies) are used to treat or prevent a disorder characterized by deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides or polypeptides, or agonists or antagonists thereof (e.g., anti-Neutrokin-alpha, and/or anti-Neutrokin-alphaSV antibodies) may be used to treat or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g.,

those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL, multiple myeloma, recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carinii.

[0646] Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention, or agonists or antagonists thereof, may be used to diagnose, prognose, treat or prevent one or more of the following diseases or disorders, or conditions associated therewith: primary immunodeficiencies, immune-mediated thrombocytopenia, Kawasaki syndrome, bone marrow transplant (e.g., recent bone marrow transplant in adults or children), chronic B-cell lymphocytic leukemia, HIV infection (e.g., adult or pediatric HIV infection), chronic inflammatory demyelinating polyneuropathy, and post-transfusion purpura.

[0647] Additionally, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention, or agonists or antagonists thereof, may be used to diagnose, prognose, treat or prevent one or more of the following diseases, disorders, or conditions associated therewith, Guillain-Barre syndrome, anemia (e.g., anemia associated with parvovirus B19, patients with stable multiple myeloma who are at high risk for infection (e.g., recurrent infection), autoimmune hemolytic anemia (e.g., warm-type autoimmune hemolytic anemia), thrombocytopenia (e.g., neonatal thrombocytopenia), and immune-mediated neutropenia), transplantation (e.g., cytomegalovirus (CMV)-negative recipients of CMV-positive organs), hypogammaglobulinemia (e.g., hypogammaglobulinemic neonates with risk factor for infection or morbidity), epilepsy (e.g., intractable epilepsy), systemic vasculitic syndromes, myasthenia gravis (e.g., decompensation in myasthenia gravis), dermatomyositis, and polymyositis.

[0648] Additional preferred embodiments of the invention include, but are not limited to, the use of Neutrokin-alpha and/or Neutrokin-alpha SV polypeptides, Neutrokin-alpha and/or Neutrokin-alpha SV polynucleotides, and functional agonists thereof, in the following applications:

[0649] Administration to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and

human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to promote or enhance immunoglobulin class switching (e.g., to induce a B cell express an IgM antibody to class switch to a different immunoglobulin isotype such as IgG, IgA, or IgE), to induce higher affinity antibody production (e.g., IgG, IgA, IgM, and IgE, for instance, by the modulation of the rate or quantity of somatic hypermutation or by modulation of the process/mechanism of selection of B cells expressing mutated antibodies), and/or to increase an immune response. In a specific nonexclusive embodiment, Neutrokin-alpha polypeptides of the invention, and/or agonists thereof, are administered to boost the immune system to produce increased quantities of IgG. In another specific nonexclusive embodiment, Neutrokin-alpha polypeptides of the invention and/or agonists thereof, are administered to boost the immune system to produce increased quantities of IgA. In another specific nonexclusive embodiment, Neutrokin-alpha polypeptides of the invention and/or agonists thereof, are administered to boost the immune system to produce increased quantities of IgM.

[0650] Administration to an animal (including, but not limited to, those listed above, and also including transgenic animals) incapable of producing functional endogenous antibody molecules or having an otherwise compromised endogenous immune system, but which is capable of producing human immunoglobulin molecules by means of a reconstituted or partially reconstituted immune system from another animal (see, e.g., published PCT Application Nos. WO98/24893, WO/9634096, WO/9633735, and WO/9110741).

[0651] A vaccine adjuvant that enhances immune responsiveness to specific antigen. In a specific embodiment, the vaccine adjuvant is a Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide described herein. In another specific embodiment, the vaccine adjuvant is a Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotide described herein (i.e., the Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotide is a genetic vaccine adjuvant). As discussed herein, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides may be administered using techniques known in the art, including but not limited to, liposomal delivery, recombinant vector delivery, injection of naked DNA, and gene gun delivery.

[0652] An adjuvant to enhance tumor-specific immune responses.

[0653] An adjuvant to enhance anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions of the invention as an adjuvant, include, but are not limited to, virus and virus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, Respiratory syncytial virus, Dengue, Rotavirus, Japanese B encephalitis, Influenza A and B, Parainfluenza, Measles, Cytomegalovirus, Rabies, Junin, Chikungunya, Rift Valley fever, Herpes simplex, and yellow fever. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to the HIV gp120 antigen.

[0654] An adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions of the invention as an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: tetanus, Diphtheria, botulism, and meningitis type B. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: Vibrio cholerae, Mycobacterium leprae, Salmonella typhi, Salmonella paratyphi, Meisseria meningitidis, Streptococcus pneumoniae, Group B streptococcus, Shigella spp., Enterotoxigenic Escherichia coli, Enterohemorrhagic E. coli, Borrelia burgdorferi, and Plasmodium (malaria).

[0655] An adjuvant to enhance anti-parasitic immune responses. Anti-parasitic immune responses that may be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a parasite. In another specific

embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to *Plasmodium* (malaria).

- [0656] As a stimulator of B cell responsiveness to pathogens.
- [0657] As an agent that elevates the immune status of an individual prior to their receipt of immunosuppressive therapies.
- [0658] As an agent to induce production of higher affinity antibodies.
- [0659] As an agent to induce class switching of B cells expressing IgM antibodies.
- [0660] As an agent to induce class switching of activated B cells expressing IgM antibodies.
- [0661] As an agent to increase serum immunoglobulin concentrations.
- [0662] As an agent to accelerate recovery of immunocompromised individuals.
- [0663] As an agent to boost immunoresponsiveness among aged populations.
- [0664] As an immune system enhancer prior to, during, or after bone marrow transplant and/or other transplants (e.g., allogeneic or xenogeneic organ transplantation). With respect to transplantation, compositions of the invention may be administered prior to, concomitant with, and/or after transplantation. In a specific embodiment, compositions of the invention are administered after transplantation, prior to the beginning of recovery of T-cell populations. In another specific embodiment, compositions of the invention are first administered after transplantation after the beginning of recovery of T cell populations, but prior to full recovery of B cell populations.
- [0665] As an agent to boost immunoresponsiveness among B cell immunodeficient individuals, such as, for example, an individual who has undergone a partial or complete splenectomy. B cell immunodeficiencies that may be ameliorated or treated by administering the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, include, but are not limited to, severe combined immunodeficiency (SCID)-X linked, SCID-autosomal, adenosine deaminase deficiency (ADA deficiency), X-linked agammaglobulinemia (XLA), Bruton's disease, congenital agammaglobulinemia, X-linked infantile agammaglobulinemia, acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, transient hypogammaglobulinemia of infancy, unspecified hypogammaglobulinemia, agammaglobulinemia, common variable immunodeficiency (CVID) (acquired), Wiskott-Aldrich Syndrome (WAS), X-linked

immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, selective IgA deficiency, IgG subclass deficiency (with or without IgA deficiency), antibody deficiency with normal or elevated IgG, immunodeficiency with thymoma, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), selective IgM immunodeficiency, recessive agammaglobulinemia (Swiss type), reticular dysgenesis, neonatal neutropenia, severe congenital leukopenia, thymic alymphoplasia-aplasia or dysplasia with immunodeficiency, ataxia-telangiectasia, short limbed dwarfism, X-linked lymphoproliferative syndrome (XLP), Nezelof syndrome-combined immunodeficiency with IgG, purine nucleoside phosphorylase deficiency (PNP), MHC Class II deficiency (Bare Lymphocyte Syndrome) and severe combined immunodeficiency.

[0666] As an agent to boost immunoresponsiveness among individuals having an acquired loss of B cell function. Conditions resulting in an acquired loss of B cell function that may be ameliorated or treated by administering the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, include, but are not limited to, HIV Infection, AIDS, bone marrow transplant, multiple myeloma and B cell chronic lymphocytic leukemia (CLL).

[0667] Patients with CLL and myeloma are at risk for increased infections. Thus, one aspect of the present invention provides for the use of Neutrokin alpha, Neutrokin alphaSV, anti-Neutrokin-alpha and or anti-Neutrokin alphaSV polynucleotides and/or polypeptides as an agent to boost immunoresponsiveness in CLL and myeloma patients.

[0668] As an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or treated by administering the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, include, but are not limited to, recovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, recovery from surgery, and recovery from burns.

[0669] As a regulator of antigen presentation by monocytes, dendritic cells, and/or B-cells. In one embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides (in soluble, membrane-bound or transmembrane forms) or polynucleotides enhance

antigen presentation or antagonize antigen presentation in vitro or in vivo. Moreover, in related embodiments, this enhancement or antagonization of antigen presentation may be useful in anti-tumor treatment or to modulate the immune system.

[0670] As a mediator of mucosal immune responses. The expression of Neutrokinne-alpha by monocytes and the responsiveness of B cells to this factor suggests that it may be involved in exchange of signals between B cells and monocytes or their differentiated progeny. This activity is in many ways analogous to the CD40-CD154 signaling between B cells and T cells. Neutrokinne-alpha may therefore be an important regulator of T cell independent immune responses to environmental pathogens. In particular, the unconventional B cell populations (CD5+) that are associated with mucosal sites and responsible for much of the innate immunity in humans may respond to Neutrokinne-alpha thereby enhancing an individual's protective immune status.

[0671] As an agent to direct an individual's immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

[0672] As a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly their susceptibility profile would likely change.

[0673] As a B cell specific binding protein to which specific activators or inhibitors of cell growth may be attached. The result would be to focus the activity of such activators or inhibitors onto normal, diseased, or neoplastic B cell populations.

[0674] As a means of detecting B-lineage cells by virtue of its specificity. This application may require labeling the protein with biotin or other agents (e.g., as described herein) to afford a means of detection.

[0675] As a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodeficiency.

[0676] As part of a B cell selection device the function of which is to isolate B cells from a heterogenous mixture of cell types. Neutrokinne-alpha could be coupled to a solid support to which B cells would then specifically bind. Unbound cells would be washed out and the bound cells subsequently eluted. A nonlimiting use of this selection would be to allow purging of tumor cells from, for example, bone marrow or peripheral blood prior to transplant.

[0677] As a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect.

[0678] As a gene-based therapy for genetically inherited disorders resulting in immuno-incompetence such as observed among SCID patients.

[0679] As an antigen for the generation of antibodies to inhibit or enhance Neutrokin-alpha mediated responses.

[0680] As a means of activating monocytes/macrophages to defend against parasitic diseases that effect monocytes such as Leshmania.

[0681] As pretreatment of bone marrow samples prior to transplant. Such treatment would increase B cell representation and thus accelerate recover.

[0682] As a means of regulating secreted cytokines that are elicited by Neutrokin-alpha.

[0683] Neutrokin-alpha or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or agonists may be used to modulate IgE concentrations in vitro or in vivo.

[0684] Additionally, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, may be used to treat, prevent, and/or diagnose IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema.

[0685] In a specific embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate selective IgA deficiency.

[0686] In another specific embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate ataxia-telangiectasia.

[0687] In another specific embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate common variable immunodeficiency.

[0688] In another specific embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate X-linked agammaglobulinemia.

[0689] In another specific embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate severe combined immunodeficiency (SCID).

[0690] In another specific embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate Wiskott-Aldrich syndrome.

[0691] In another specific embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate X-linked Ig deficiency with hyper IgM.

[0692] In another specific embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or agonists or antagonists (e.g., anti-Neutrokin-alpha antibodies) thereof, is administered to treat, prevent, and/or diagnose chronic myelogenous leukemia, acute myelogenous leukemia, leukemia, hystiocytic leukemia, monocytic leukemia (e.g., acute monocytic leukemia), leukemic reticulosis, Shilling Type monocytic leukemia, and/or other leukemias derived from monocytes and/or monocytic cells and/or tissues.

[0693] In another specific embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate monocytic leukemoid reaction, as seen, for example, with tuberculosis.

[0694] In another specific embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or agonists thereof, is administered to treat, prevent, diagnose, and/or ameliorate monocytic leukocytosis, monocytic leukopenia, monocytopenia, and/or monocytosis.

[0695] In a specific embodiment, Neutrokin-alpha, and Neutrokin-alphaSV polynucleotides or polypeptides of the invention, and/or anti-Neutrokin-alpha antibodies and/or agonists or antagonists thereof, are used to treat, prevent, detect, and/or diagnose primary B lymphocyte disorders and/or diseases, and/or conditions associated therewith. In one embodiment, such primary B lymphocyte disorders, diseases, and/or conditions are characterized by a complete or partial loss of humoral immunity. Primary B lymphocyte disorders, diseases, and/or conditions associated therewith that are characterized by a complete or partial loss of humoral immunity and that may be prevented, treated, detected

and/or diagnosed with compositions of the invention include, but are not limited to, X-Linked Agammaglobulinemia (XLA), severe combined immunodeficiency disease (SCID), and selective IgA deficiency.

[0696] In a preferred embodiment, Neutrokin-alpha and Neutrokin-alphaSV polynucleotides, polypeptides, and/or agonists and/or antagonists thereof are used to treat, prevent, and/or diagnose diseases or disorders affecting or conditions associated with any one or more of the various mucous membranes of the body. Such diseases or disorders include, but are not limited to, for example, mucositis, mucoclasia, mucocolitis, mucocutaneous leishmaniasis (such as, for example, American leishmaniasis, leishmaniasis americana, nasopharyngeal leishmaniasis, and New World leishmaniasis), mucocutaneous lymph node syndrome (for example, Kawasaki disease), mucoenteritis, mucoepidermoid carcinoma, mucoepidermoid tumor, mucoepithelial dysplasia, mucoid adenocarcinoma, mucoid degeneration, myxoid degeneration; myxomatous degeneration; myxomatosis, mucoid medial degeneration (for example, cystic medial necrosis), mucolipidosis (including, for example, mucolipidosis I, mucolipidosis II, mucolipidosis III, and mucolipidosis IV), mucolysis disorders, mucomembranous enteritis, mucoenteritis, mucopolysaccharidosis (such as, for example, type I mucopolysaccharidosis (i.e., Hurler's syndrome), type IS mucopolysaccharidosis (i.e., Scheie's syndrome or type V mucopolysaccharidosis), type II mucopolysaccharidosis (i.e., Hunter's syndrome), type III mucopolysaccharidosis (i.e., Sanfilippo's syndrome), type IV mucopolysaccharidosis (i.e., Morquio's syndrome), type VI mucopolysaccharidosis (i.e., Maroteaux-Lamy syndrome), type VII mucopolysaccharidosis (i.e., mucopolysaccharidosis due to beta-glucuronidase deficiency), and mucosulfatidosis), mucopolysacchariduria, mucopurulent conjunctivitis, mucopus, mucormycosis (i.e., zygomycosis), mucosal disease (i.e., bovine virus diarrhea), mucous colitis (such as, for example, mucocolitis and myxomembranous colitis), and mucoviscidosis (such as, for example, cystic fibrosis, cystic fibrosis of the pancreas, Clarke-Hadfield syndrome, fibrocystic disease of the pancreas, mucoviscidosis, and viscidosis). In a highly preferred embodiment, Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides, polypeptides, and/or agonists and/or antagonists thereof are used to treat, prevent, and/or diagnose mucositis, especially as associated with chemotherapy.

[0697] In a preferred embodiment, Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides, polypeptides, and/or agonists and/or antagonists thereof are used to treat, prevent, and/or diagnose diseases or disorders affecting or conditions associated with sinusitis.

[0698] An additional condition, disease or symptom that can be treated, prevented, and/or diagnosed by Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides or polypeptides, or agonists of Neutrokin-alpha, and/or Neutrokin-alphaSV, is osteomyelitis.

[0699] An additional condition, disease or symptom that can be treated, prevented, and/or diagnosed by Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides or polypeptides, or agonists of Neutrokin-alpha, and/or Neutrokin-alphaSV, is endocarditis.

[0700] All of the above described applications as they may apply to veterinary medicine.

[0701] Antagonists of Neutrokin-alpha include binding and/or inhibitory antibodies, antisense nucleic acids, ribozymes, and Neutrokin-alpha polypeptides of the invention. These would be expected to reverse many of the activities of the ligand described above as well as find clinical or practical application as:

[0702] A means of blocking various aspects of immune responses to foreign agents or self. Examples include autoimmune disorders such as lupus, and arthritis, as well as immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and pathogens. Although our current data speaks directly to the potential role of Neutrokin-alpha in B cell and monocyte related pathologies, it remains possible that other cell types may gain expression or responsiveness to Neutrokin-alpha. Thus, Neutrokin-alpha may, like CD40 and its ligand, be regulated by the status of the immune system and the microenvironment in which the cell is located.

[0703] A therapy for preventing the B cell proliferation and Ig secretion associated with autoimmune diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythematosus and MS.

[0704] An inhibitor of graft versus host disease or transplant rejection.

[0705] A therapy for B cell malignancies such as ALL, Hodgkins disease, non-Hodgkins lymphoma, Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, and EBV-transformed diseases.

[0706] A therapy for chronic hypergammaglobulinemeia evident in such diseases as monoclonal gammopathy of undetermined significance (MGUS), Waldenstrom's disease, related idiopathic monoclonal gammopathies, and plasmacytomas.

[0707] A therapy for decreasing cellular proliferation of Large B-cell Lymphomas.

[0708] A means of decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.

[0709] An immunosuppressive agent(s).

[0710] Neutrokin-alpha or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or antagonists may be used to modulate IgE concentrations in vitro or in vivo.

[0711] In another embodiment, administration of Neutrokin-alpha or Neutrokin-alphaSV polypeptides or polynucleotides of the invention, or antagonists thereof, may be used to treat, prevent, and/or diagnose IgE-mediated allergic reactions including, but not limited to, asthma, rhinitis, and eczema.

[0712] An inhibitor of signaling pathways involving ERK1, COX2 and Cyclin D2 which have been associated with Neutrokin-alpha induced B cell activation.

[0713] The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

[0714] The agonists and antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described herein.

[0715] The antagonists may be employed for instance to inhibit Neutrokin-alpha-mediated and/or Neutrokin-alphaSV-mediated chemotaxis and activation of macrophages and their precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated and CD8 cytotoxic T cells and natural killer cells, in certain auto-immune and chronic inflammatory and infective diseases. Examples of auto-immune

diseases include multiple sclerosis, and insulin-dependent diabetes. The antagonists may also be employed to treat, prevent, and/or diagnose infectious diseases including silicosis, sarcoidosis, idiopathic pulmonary fibrosis by preventing the recruitment and activation of mononuclear phagocytes. They may also be employed to treat, prevent, and/or diagnose idiopathic hyper-eosinophilic syndrome by preventing eosinophil production and migration. Endotoxic shock may also be treated by the antagonists by preventing the migration of macrophages and their production of the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the present invention. The antagonists may also be employed for treating atherosclerosis, by preventing monocyte infiltration in the artery wall. The antagonists may also be employed to treat, prevent, and/or diagnose histamine-mediated allergic reactions and immunological disorders including late phase allergic reactions, chronic urticaria, and atopic dermatitis by inhibiting chemokine-induced mast cell and basophil degranulation and release of histamine. IgE-mediated allergic reactions such as allergic asthma, rhinitis, and eczema may also be treated. The antagonists may also be employed to treat, prevent, and/or diagnose chronic and acute inflammation by preventing the attraction of monocytes to a wound area. They may also be employed to regulate normal pulmonary macrophage populations, since chronic and acute inflammatory pulmonary diseases are associated with sequestration of mononuclear phagocytes in the lung. Antagonists may also be employed to treat, prevent, and/or diagnose rheumatoid arthritis by preventing the attraction of monocytes into synovial fluid in the joints of patients. Monocyte influx and activation plays a significant role in the pathogenesis of both degenerative and inflammatory arthropathies. The antagonists may be employed to interfere with the deleterious cascades attributed primarily to IL-1 and TNF, which prevents the biosynthesis of other inflammatory cytokines. In this way, the antagonists may be employed to prevent inflammation. The antagonists may also be employed to inhibit prostaglandin-independent fever induced by Neutrokin-alpha and/or Neutrokin-alphaSV. The antagonists may also be employed to treat, prevent, and/or diagnose cases of bone marrow failure, for example, aplastic anemia and myelodysplastic syndrome. The antagonists may also be employed to treat, prevent, and/or diagnose asthma and allergy by preventing eosinophil accumulation in the lung. The antagonists may also be employed to treat, prevent, and/or diagnose subepithelial basement membrane fibrosis which is a prominent feature of the asthmatic lung. The antagonists may also be

employed to treat, prevent, and/or diagnose lymphomas (e.g., one or more of the extensive, but not limiting, list of lymphomas provided herein).

[0716] All of the above described applications as they may apply to veterinary medicine. Moreover, all applications described herein may also apply to veterinary medicine.

[0717] Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, may be used to treat, prevent, and/or diagnose various immune system-related disorders and/or conditions associated with these disorders, in mammals, preferably humans. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof that can inhibit an immune response, particularly the proliferation of B cells and/or the production of immunoglobulins, may be an effective therapy in treating and/or preventing autoimmune disorders. Thus, in preferred embodiments, Neutrokine-alpha and/or Neutrokine-alphaSV antagonists of the invention (e.g., polypeptide fragments of Neutrokine-alpha and/or Neutrokine-alphaSV and anti-Neutrokine-alpha antibodies) are used to treat, prevent, and/or diagnose an autoimmune disorder.

[0718] Autoimmune disorders and conditions associated with these disorders that may be treated, prevented, and/or diagnosed with the Neutrokine-alpha polynucleotides, polypeptides, and/or antagonists of the invention (e.g., anti-Neutrokine-alpha antibodies), include, but are not limited to, autoimmune hemolytic anemia, autoimmune neutropenia, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, autoimmune neutropenia, hemolytic anemia, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, glomerulonephritis (e.g., IgA nephropathy), dense deposit disease, Multiple Sclerosis, Neuritis, Uveitis Ophthalmia, Polyendocrinopathies, Purpura (e.g., Henoch-Schoenlein purpura), Reiter's Disease, Stiff-Man Syndrome, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, gluten sensitive enteropathy, insulin dependent diabetes mellitus, discoid lupus, and autoimmune inflammatory eye disease.

[0719] Additional autoimmune disorders (that are highly probable) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, autoimmune thyroiditis, hypothyroidism (i.e., Hashimoto's thyroiditis) (often characterized, e.g., by cell-mediated and humoral thyroid cytotoxicity), systemic lupus erythematosus (often characterized, e.g., by circulating and locally generated immune complexes), Goodpasture's syndrome (often characterized, e.g., by anti-basement membrane antibodies), Pemphigus (often characterized, e.g., by epidermal acantholytic antibodies), Receptor autoimmunities such as, for example, (a) Graves' Disease (often characterized, e.g., by TSH receptor antibodies), (b) Myasthenia Gravis (often characterized, e.g., by acetylcholine receptor antibodies), and (c) insulin resistance (often characterized, e.g., by insulin receptor antibodies), autoimmune hemolytic anemia (often characterized, e.g., by phagocytosis of antibody-sensitized RBCs), autoimmune thrombocytopenic purpura (often characterized, e.g., by phagocytosis of antibody-sensitized platelets).

[0720] Additional autoimmune disorders (that are probable) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, rheumatoid arthritis (often characterized, e.g., by immune complexes in joints), scleroderma with anti-collagen antibodies (often characterized, e.g., by nucleolar and other nuclear antibodies), mixed connective tissue disease (often characterized, e.g., by antibodies to extractable nuclear antigens (e.g., ribonucleoprotein)), polymyositis/dermatomyositis (often characterized, e.g., by nonhistone ANA), pernicious anemia (often characterized, e.g., by antiparietal cell, microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility (often characterized, e.g., by antispermatozoal antibodies), glomerulonephritis (often characterized, e.g., by glomerular basement membrane antibodies or immune complexes) such as primary glomerulonephritis and IgA nephropathy, bullous pemphigoid (often characterized, e.g., by IgG and complement in basement membrane), Sjogren's syndrome (often characterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes mellitus (often characterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies).

[0721] Additional autoimmune disorders (that are possible) that may be treated, prevented, and/or diagnosed with the compositions of the invention include, but are not limited to, chronic active hepatitis (often characterized, e.g., by smooth muscle antibodies), primary biliary cirrhosis (often characterized, e.g., by mitochondrial antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some cases), vitiligo (often characterized, e.g., by melanocyte antibodies), vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by myocardial antibodies), cardiotomy syndrome (often characterized, e.g., by myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma (often characterized, e.g., by IgG and IgM antibodies to IgE), inflammatory myopathies, and many other inflammatory, granulomatous, degenerative, and atrophic disorders.

[0722] In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, and/or diagnosed using anti-Neutrokin-alpha antibodies and/or anti-Neutrokin-alphaSV.

[0723] In a specific preferred embodiment, rheumatoid arthritis is treated, prevented, and/or diagnosed using anti-Neutrokin-alpha antibodies and/or anti-Neutrokin-alphaSV antibodies and/or other antagonist of the invention.

[0724] In a specific preferred embodiment, lupus is treated, prevented, and/or diagnosed using anti-Neutrokin-alpha antibodies and/or anti-Neutrokin-alphaSV antibodies and/or other antagonist of the invention.

[0725] In a specific preferred embodiment, Sjögren's Syndrome is treated, prevented, and/or diagnosed using anti-Neutrokin-alpha antibodies and/or anti-Neutrokin-alphaSV antibodies and/or other antagonist of the invention.

[0726] In a specific preferred embodiment, AIDS is treated, prevented, and/or diagnosed using anti-Neutrokin-alpha antibodies and/or anti-Neutrokin-alphaSV antibodies and/or other antagonist of the invention.

[0727] In a specific preferred embodiment, HIV infection is treated, prevented, and/or diagnosed using anti-Neutrokin-alpha antibodies and/or anti-Neutrokin-alphaSV antibodies and/or other antagonist of the invention.

[0728] In a specific preferred embodiment, nephritis associated with lupus is treated, prevented, and/or diagnosed using anti-Neutrokin-alpha antibodies and/or anti-Neutrokin-alphaSV antibodies and/or other antagonist of the invention.

[0729] In a specific embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides, or antagonists thereof (e.g., anti-Neutrokin-alpha and/or anti-Neutrokin-alphaSV antibodies) are used to treat or prevent systemic lupus erythematosus and/or diseases, disorders or conditions associated therewith. Lupus-associated diseases, disorders, or conditions that may be treated or prevented with Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides, or antagonists of the invention, include, but are not limited to, hematologic disorders (e.g., hemolytic anemia, leukopenia, lymphopenia, and thrombocytopenia), immunologic disorders (e.g., anti-DNA antibodies, and anti-Sm antibodies), rashes, photosensitivity, oral ulcers, arthritis, fever, fatigue, weight loss, serositis (e.g., pleuritus (pleurisy)), renal disorders (e.g., nephritis), neurological disorders (e.g., seizures, peripheral neuropathy, CNS related disorders), gastrointestinal disorders, Raynaud phenomenon, and pericarditis. In a preferred embodiment, the Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides, or antagonists thereof (e.g., anti-Neutrokin-alpha and/or anti-Neutrokin-alphaSV antibodies) are used to treat or prevent renal disorders associated with systemic lupus erythematosus. In a most preferred embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides, or antagonists thereof (e.g., anti-Neutrokin-alpha and/or anti-Neutrokin-alphaSV antibodies) are used to treat or prevent nephritis associated with systemic lupus erythematosus.

[0730] Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof. Moreover, these molecules can be used to treat, prevent, and/or diagnose anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

[0731] Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, may also be used to treat, prevent, and/or diagnose organ rejection or graft-versus-host disease (GVHD) and/or conditions associated therewith. Organ rejection occurs by host immune cell destruction

of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

[0732] Similarly, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, may also be used to modulate inflammation. For example, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat, prevent, and/or diagnose inflammatory conditions, both chronic and acute conditions, including chronic prostatitis, granulomatous prostatitis and malacoplakia, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

[0733] In a specific embodiment, anti-Neutrokin-alpha antibodies and/or anti-Neutrokin-alphaSV antibodies of the invention are used to treat, prevent, modulate, detect, and/or diagnose inflammation.

[0734] In a specific embodiment, anti-Neutrokin-alpha antibodies and/or anti-Neutrokin-alphaSV antibodies of the invention are used to treat, prevent, modulate, detect, and/or diagnose inflammatory disorders.

[0735] In another specific embodiment, anti-Neutrokin-alpha antibodies and/or anti-Neutrokin-alphaSV antibodies of the invention are used to treat, prevent, modulate, detect, and/or diagnose allergy and/or hypersensitivity.

[0736] In another embodiment, therapeutic or pharmaceutical compositions of the invention (e.g., Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention and/or antagonists thereof) are administered to an animal to

treat, prevent or ameliorate ischemia and arteriosclerosis. Examples of such disorders include, but are not limited to, reperfusion damage (e.g., in the heart and/or brain) and cardiac hypertrophy.

[0737] Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention and/or antagonists thereof, may also be used to modulate blood clotting and to treat or prevent blood clotting disorders, such as, for example, antibody-mediated thrombosis (i.e., antiphospholipid antibody syndrome (APS)). For example, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention and/or antagonists thereof, may inhibit the proliferation and differentiation of cells involved in producing anticardiolipin antibodies. These compositions of the invention can be used to treat, prevent, and/or diagnose, thrombotic related events including, but not limited to, stroke (and recurrent stroke), heart attack, deep vein thrombosis, pulmonary embolism, myocardial infarction, coronary artery disease (e.g., antibody -mediated coronary artery disease), thrombosis, graft reocclusion following cardiovascular surgery (e.g., coronary arterial bypass grafts, recurrent fetal loss, and recurrent cardiovascular thromboembolic events.

[0738] Antibodies against Neutrokin-alpha and/or Neutrokin-alphaSV may be employed to bind to and inhibit Neutrokin-alpha and/or Neutrokin-alphaSV activity to treat, prevent, and/or diagnose ARDS, by preventing infiltration of neutrophils into the lung after injury. The agonists and antagonists of the instant may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as described hereinafter.

[0739] Neutrokin-alpha and/or Neutrokin-alphaSV and/or Neutrokin-alpha receptor polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, are used to treat, prevent, and/or diagnose diseases and disorders of the pulmonary system (e.g., bronchi such as, for example, sinopulmonary and bronchial infections and conditions associated with such diseases and disorders and other respiratory diseases and disorders. In specific embodiments, such diseases and disorders include, but are not limited to, bronchial adenoma, bronchial asthma, pneumonia (such as, e.g., bronchial pneumonia, bronchopneumonia, and tuberculous bronchopneumonia), chronic obstructive pulmonary disease (COPD), bronchial polyps, bronchiectasia (such as, e.g., bronchiectasia sicca, cylindrical bronchiectasis, and saccular bronchiectasis), bronchiolar adenocarcinoma, bronchiolar carcinoma, bronchiolitis (such as, e.g., exudative

bronchiolitis, bronchiolitis fibrosa obliterans, and proliferative bronchiolitis), bronchioloalveolar carcinoma, bronchitic asthma, bronchitis (such as, e.g., asthmatic bronchitis, Castellani's bronchitis, chronic bronchitis, croupous bronchitis, fibrinous bronchitis, hemorrhagic bronchitis, infectious avian bronchitis, obliterative bronchitis, plastic bronchitis, pseudomembranous bronchitis, putrid bronchitis, and verminous bronchitis), bronchocentric granulomatosis, bronchoedema, bronchoesophageal fistula, bronchogenic carcinoma, bronchogenic cyst, broncholithiasis, bronchomalacia, bronchomycosis (such as, e.g., bronchopulmonary aspergillosis), bronchopulmonary spirochetosis, hemorrhagic bronchitis, bronchorrhea, bronchospasm, bronchostaxis, bronchostenosis, Biot's respiration, bronchial respiration, Kussmaul respiration, Kussmaul-Kien respiration, respiratory acidosis, respiratory alkalosis, respiratory distress syndrome of the newborn, respiratory insufficiency, respiratory scleroma, respiratory syncytial virus, and the like.

[0740] In a specific embodiment, Neutrokinne-alpha and/or Neutrokinne-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, are used to treat, prevent, and/or diagnose chronic obstructive pulmonary disease (COPD).

[0741] In another embodiment, Neutrokinne-alpha and/or Neutrokinne-alphaSV polynucleotides or polypeptides of the invention and/or agonists and/or antagonists thereof, are used to treat, prevent, and/or diagnose fibroses and conditions associated with fibroses, such as, for example, but not limited to, cystic fibrosis (including such fibroses as cystic fibrosis of the pancreas, Clarke-Hadfield syndrome, fibrocystic disease of the pancreas, mucoviscidosis, and viscidosis), endomyocardial fibrosis, idiopathic retroperitoneal fibrosis, leptomeningeal fibrosis, mediastinal fibrosis, nodular subepidermal fibrosis, pericentral fibrosis, perimuscular fibrosis, pipestem fibrosis, replacement fibrosis, subadventitial fibrosis, and Symmers' clay pipestem fibrosis.

[0742] The TNF family ligands are known to be among the most pleiotropic cytokines, inducing a large number of cellular responses, including cytotoxicity, anti-viral activity, immunoregulatory activities, and the transcriptional regulation of several genes (D.V. Goeddel *et al.*, "Tumor Necrosis Factors: Gene Structure and Biological Activities," *Symp. Quant. Biol.* 51:597- 609 (1986), Cold Spring Harbor; B. Beutler and A. Cerami, *Annu. Rev. Biochem.* 57:505-518 (1988); L.J. Old, *Sci. Am.* 258:59-75 (1988); W. Fiers, *FEBS Lett.* 285:199-224 (1991)). The TNF-family ligands, including Neutrokinne-alpha

and/or Neutrokine-alphaSV of the present invention, induce such various cellular responses by binding to TNF-family receptors. Neutrokine-alpha and/or Neutrokine-alphaSV polypeptides are believed to elicit a potent cellular response including any genotypic, phenotypic, and/or morphologic change to the cell, cell line, tissue, tissue culture or patient. As indicated, such cellular responses include not only normal physiological responses to TNF-family ligands, but also diseases associated with increased apoptosis or the inhibition of apoptosis. Apoptosis-programmed cell death is a physiological mechanism involved in the deletion of peripheral B and/or T lymphocytes of the immune system, and its disregulation can lead to a number of different pathogenic processes (J.C. Ameisen, *AIDS* 8:1197-1213 (1994); P.H. Krammer *et al.*, *Curr. Opin. Immunol.* 6:279-289 (1994)).

[0743] Diseases associated with increased cell survival, or the inhibition of apoptosis that may be diagnosed, treated, or prevented with the Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention, and agonists and antagonists thereof, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to, colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as systemic lupus erythematosus and immune-related glomerulonephritis, rheumatoid arthritis); viral infections (such as herpes viruses, pox viruses and adenoviruses); inflammation; graft vs. host disease; acute graft rejection and chronic graft rejection. Thus, in preferred embodiments Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention and/or agonists or antagonists thereof, are used to treat, prevent, and/or diagnose autoimmune diseases and/or inhibit the growth, progression, and/or metastasis of cancers, including, but not limited to, those cancers disclosed herein, such as, for example, lymphocytic leukemias (including, for example, MLL and chronic lymphocytic leukemia (CLL)) and follicular lymphomas. In another embodiment Neutrokine-alpha and/or Neutrokine-alphaSV polynucleotides or polypeptides of the invention are used to activate, differentiate or proliferate cancerous cells or tissue (e.g., B cell lineage related cancers (e.g., CLL and MLL), lymphocytic

leukemia, or lymphoma) and thereby render the cells more vulnerable to cancer therapy (e.g., chemotherapy or radiation therapy).

[0744] Moreover, in other embodiments, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention or agonists or antagonists thereof, are used to inhibit the growth, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendrogioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

[0745] In specific embodiments Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention are used to inhibit the growth, progression, and/or metastases of multiple myeloma. In even more specific embodiments, radiolabeled Neutrokin-alpha polypeptides comprising, or alternatively consisting of amino acids 134-285 of SEQ ID NO:2 (e.g., radiolabeled Neutrokin alpha trimers comprising three polypeptide chains consisting of amino acids 134-285 of SEQ ID NO:2) are used to inhibit the growth, progression, and/or metastases of multiple myeloma. In

particular embodiments, the radiolabeled Neutrokin-alpha polypeptides are radiolabeled with an ¹³¹I radioisotope.

[0746] In specific embodiments Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention are used to inhibit the growth, progression, and/or metastases of non-Hodgkin's lymphoma. In even more specific embodiments, radiolabeled Neutrokin-alpha polypeptides comprising, or alternatively consisting of amino acids 134-285 of SEQ ID NO:2 (e.g., radiolabeled Neutrokin alpha trimers comprising three polypeptide chains consisting of amino acids 134-285 of SEQ ID NO:2) are used to inhibit the growth, progression, and/or metastases of non-Hodgkin's lymphoma. In particular embodiments, the radiolabeled Neutrokin-alpha polypeptides are radiolabeled with an ¹³¹I radioisotope.

[0747] In specific embodiments Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention are used to inhibit the growth, progression, and/or metastases of chronic lymphocytic leukemia (CLL). In even more specific embodiments, radiolabeled Neutrokin-alpha polypeptides comprising, or alternatively consisting of amino acids 134-285 of SEQ ID NO:2 (e.g., radiolabeled Neutrokin alpha trimers comprising three polypeptide chains consisting of amino acids 134-285 of SEQ ID NO:2) are used to inhibit the growth, progression, and/or metastases of CLL. In particular embodiments, the radiolabeled Neutrokin-alpha polypeptides are radiolabeled with an ¹³¹I radioisotope.

[0748] Diseases associated with increased apoptosis that may be diagnosed, treated, or prevented with the Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention, and agonists and antagonists thereof, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration); myelodysplastic syndromes (such as aplastic anemia), ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia. Thus, in preferred embodiments Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention and/or agonists or antagonists thereof, are used to treat, prevent, and/or diagnose the diseases and disorders listed above.

[0749] In preferred embodiments, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention and/or agonists or antagonists thereof (e.g., anti-Neutrokin-alpha antibodies) inhibit the growth of human histiocytic lymphoma U-937 cells in a dose-dependent manner. In additional preferred embodiments, Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention and/or agonists or antagonists thereof (e.g., anti-Neutrokin-alpha antibodies) inhibit the growth of PC-3 cells, HT-29 cells, HeLa cells, MCF-7 cells, and A293 cells. In highly preferred embodiments, Neutrokin-alpha and/or Neutrokin-alphaSV polynucleotides or polypeptides of the invention and/or agonists or antagonists thereof (e.g., anti-Neutrokin-alpha antibodies) are used to inhibit growth, progression, and/or metastasis of prostate cancer, colon cancer, cervical carcinoma, and breast carcinoma.

[0750] Thus, in additional preferred embodiments, the present invention is directed to a method for enhancing apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses a Neutrokin-alpha and/or Neutrokin-alphaSV receptor an effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV, or an agonist or antagonist thereof, capable of increasing or decreasing Neutrokin-alpha and/or Neutrokin-alphaSV mediated signaling. Preferably, Neutrokin-alpha and/or Neutrokin-alphaSV mediated signaling is increased or decreased to treat, prevent, and/or diagnose a disease wherein decreased apoptosis or decreased cytokine and adhesion molecule expression is exhibited. An agonist or antagonist can include soluble forms of Neutrokin-alpha and/or Neutrokin-alphaSV and monoclonal antibodies directed against the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide.

[0751] In a further aspect, the present invention is directed to a method for inhibiting apoptosis induced by a TNF-family ligand, which involves administering to a cell which expresses the Neutrokin-alpha and/or Neutrokin-alphaSV receptor an effective amount of an agonist or antagonist capable of increasing or decreasing Neutrokin-alpha and/or Neutrokin-alphaSV mediated signaling. Preferably, Neutrokin-alpha and/or Neutrokin-alphaSV mediated signaling is increased or decreased to treat, prevent, and/or diagnose a disease wherein increased apoptosis or NF-kappaB expression is exhibited. An agonist or antagonist can include soluble forms of Neutrokin-alpha and/or Neutrokin-alphaSV and monoclonal antibodies directed against the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide.

[0752] Because Neutrokin-alpha and/or Neutrokin-alphaSV belong to the TNF superfamily, the polypeptides should also modulate angiogenesis. In addition, since Neutrokin-alpha and/or Neutrokin-alphaSV inhibit immune cell functions, the polypeptides will have a wide range of anti-inflammatory activities. Neutrokin-alpha and/or Neutrokin-alphaSV may be employed as an anti-neovascularizing agent to treat, prevent, and/or diagnose solid tumors by stimulating the invasion and activation of host defense cells, e.g., cytotoxic T cells and macrophages and by inhibiting the angiogenesis of tumors. Those of skill in the art will recognize other non-cancer indications where blood vessel proliferation is not wanted. They may also be employed to enhance host defenses against resistant chronic and acute infections, for example, myobacterial infections via the attraction and activation of microbicidal leukocytes. Neutrokin-alpha and/or Neutrokin-alphaSV may also be employed to inhibit T-cell proliferation by the inhibition of IL-2 biosynthesis for the treatment of T-cell mediated auto-immune diseases and lymphocytic leukemias (including, for example, chronic lymphocytic leukemia (CLL)). Neutrokin-alpha and/or Neutrokin-alphaSV may also be employed to stimulate wound healing, both via the recruitment of debris clearing and connective tissue promoting inflammatory cells. In this same manner, Neutrokin-alpha and/or Neutrokin-alphaSV may also be employed to treat, prevent, and/or diagnose other fibrotic disorders, including liver cirrhosis, osteoarthritis and pulmonary fibrosis. Neutrokin-alpha and/or Neutrokin-alphaSV also increases the presence of eosinophils that have the distinctive function of killing the larvae of parasites that invade tissues, as in schistosomiasis, trichinosis and ascariasis. It may also be employed to regulate hematopoiesis, by regulating the activation and differentiation of various hematopoietic progenitor cells, for example, to release mature leukocytes from the bone marrow following chemotherapy, i.e., in stem cell mobilization. Neutrokin-alpha and/or Neutrokin-alphaSV may also be employed to treat, prevent, and/or diagnose sepsis.

[0753] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in the diagnosis and treatment or prevention of a wide range of diseases and/or conditions. Such diseases and conditions include, but are not limited to, cancer (e.g., immune cell related cancers, breast cancer, prostate cancer, ovarian cancer, follicular lymphoma, cancer associated with mutation or alteration of p53, brain tumor, bladder cancer, uterocervical cancer, colon cancer, colorectal cancer, non-small cell

carcinoma of the lung, small cell carcinoma of the lung, stomach cancer, etc.), lymphoproliferative disorders (e.g., lymphadenopathy), microbial (e.g., viral, bacterial, etc.) infection (e.g., HIV-1 infection, HIV-2 infection, herpesvirus infection (including, but not limited to, HSV-1, HSV-2, CMV, VZV, HHV-6, HHV-7, EBV), adenovirus infection, poxvirus infection, human papilloma virus infection, hepatitis infection (e.g., HAV, HBV, HCV, etc.), Helicobacter pylori infection, invasive Staphylococcia, etc.), parasitic infection, nephritis, bone disease (e.g., osteoporosis), atherosclerosis, pain, cardiovascular disorders (e.g., neovascularization, hypovascularization or reduced circulation (e.g., ischemic disease (e.g., myocardial infarction, stroke, etc.)), AIDS, allergy, inflammation, neurodegenerative disease (e.g., Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, pigmentary retinitis, cerebellar degeneration, etc.), graft rejection (acute and chronic), graft vs. host disease, diseases due to osteomyelodysplasia (e.g., aplastic anemia, etc.), joint tissue destruction in rheumatism, liver disease (e.g., acute and chronic hepatitis, liver injury, and cirrhosis), autoimmune disease (e.g., multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, immune complex glomerulonephritis, autoimmune diabetes, autoimmune thrombocytopenic purpura, Grave's disease, Hashimoto's thyroiditis, etc.), cardiomyopathy (e.g., dilated cardiomyopathy), diabetes, diabetic complications (e.g., diabetic nephropathy, diabetic neuropathy, diabetic retinopathy), influenza, asthma, psoriasis, glomerulonephritis, septic shock, and ulcerative colitis.

[0754] Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in promoting angiogenesis, wound healing (e.g., wounds, burns, and bone fractures). Polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are also useful as an adjuvant to enhance immune responsiveness to specific antigen, anti-viral immune responses.

[0755] More generally, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists thereof are useful in regulating (i.e., elevating or reducing) immune response. For example, polynucleotides and/or polypeptides of the invention may be useful in preparation or recovery from surgery, trauma, radiation therapy, chemotherapy, and transplantation, or may be used to boost immune response and/or recovery in the elderly and immunocompromised individuals. Alternatively, polynucleotides and/or polypeptides of the invention and/or agonists and/or antagonists

thereof are useful as immunosuppressive agents, for example in the treatment or prevention of autoimmune disorders. In specific embodiments, polynucleotides and/or polypeptides of the invention are used to treat or prevent chronic inflammatory, allergic or autoimmune conditions, such as those described herein or are otherwise known in the art.

[0756] Preferably, treatment using Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotides or polypeptides, and/or agonists or antagonists of Neutrokin-alpha, and/or Neutrokin-alphaSV (e.g., anti-Neutrokin-alpha antibody), could either be by administering an effective amount of Neutrokin-alpha, and/or Neutrokin-alphaSV polypeptide of the invention, or agonist or antagonist thereof, to the patient, or by removing cells from the patient, supplying the cells with Neutrokin-alpha, and/or Neutrokin-alphaSV polynucleotide, and returning the engineered cells to the patient (ex vivo therapy). Moreover, as further discussed herein, the Neutrokin-alpha, and/or Neutrokin-alphaSV polypeptide or polynucleotide can be used as an adjuvant in a vaccine to raise an immune response against infectious disease.

Formulations and Administration

[0757] The Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide composition (preferably containing a polypeptide which is a soluble form of the Neutrokin-alpha and/or Neutrokin-alphaSV extracellular domains) will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide alone), the site of delivery of the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide for purposes herein is thus determined by such considerations.

[0758] As a general proposition, the total pharmaceutically effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide administered parenterally per dose will be in the range of about 1 microgram/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day.

[0759] In another embodiment, the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide of the invention is administered to a human at a dose between 0.0001 and 0.045 mg/kg/day, preferably, at a dose between 0.0045 and 0.045 mg/kg/day, and more preferably, at a dose of about 45 microgram/kg/day in humans; and at a dose of about 3 mg/kg/day in mice.

[0760] If given continuously, the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide is typically administered at a dose rate of about 1 microgram/kg/hour to about 50 micrograms/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed.

[0761] The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

[0762] In a specific embodiment, the total pharmaceutically effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide administered parenterally per dose will be in the range of about 0.1 microgram/kg/day to 45 micrograms/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.1 microgram/kg/day, and most preferably for humans between about 0.01 and 50 micrograms/kg/day for the protein. Neutrokin-alpha and/or Neutrokin-alphaSV may be administered as a continuous infusion, multiple discreet injections per day (e.g., three or more times daily, or twice daily), single injection per day, or as discreet injections given intermittently (e.g., twice daily, once daily, every other day, twice weekly, weekly, biweekly, monthly, bimonthly, and quarterly). If given continuously, the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide is typically administered at a dose rate of about 0.001 to 10 microgram/kg/hour to about 50 micrograms/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump.

[0763] Effective dosages of the compositions of the present invention to be administered may be determined through procedures well known to those in the art which address such parameters as biological half-life, bioavailability, and toxicity. Such determination is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0764] Bioexposure of an organism to Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide during therapy may also play an important role in determining a therapeutically and/or pharmacologically effective dosing regime. Variations of dosing such as repeated administrations of a relatively low dose of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide for a relatively long period of time may have an effect which is therapeutically and/or pharmacologically distinguishable from that achieved with repeated administrations of a relatively high dose of Neutrokin-alpha and/or Neutrokin-alphaSV for a relatively short period of time. See, for instance, the serum immunoglobulin level experiments presented in Example 6.

[0765] Using the equivalent surface area dosage conversion factors supplied by Freireich, E. J., et al. (*Cancer Chemotherapy Reports* 50(4):219-44 (1966)), one of ordinary skill in the art is able to conveniently convert data obtained from the use of Neutrokin-alpha and/or Neutrokin-alphaSV in a given experimental system into an accurate estimation of a pharmaceutically effective amount of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide to be administered per dose in another experimental system. Experimental data obtained through the administration of Neutrokin-alpha in mice (see, for instance, Example 6) may be converted through the conversion factors supplied by Freireich, et al., to accurate estimates of pharmaceutically effective doses of Neutrokin-alpha in rat, monkey, dog, and human. The following conversion table (Table III) is a summary of the data provided by Freireich, et al. Table III gives approximate factors for converting doses expressed in terms of mg/kg from one species to an equivalent surface area dose expressed as mg/kg in another species tabulated.

Table III. Equivalent Surface Area Dosage Conversion Factors.

		--TO--		
		Mouse	Rat	Monkey
--FROM-- (20g)		(150g)	(3.5kg)	(8kg)
Mouse	1	1/2	1/4	1/6
Rat	2	1	1/2	1/4
Monkey	4	2	1	3/5
Dog	6	4	5/3	1
Human	12	7	3	2
				1

[0766] Thus, for example, using the conversion factors provided in Table III, a dose of 50 mg/kg in the mouse converts to an appropriate dose of 12.5 mg/kg in the monkey because $(50 \text{ mg/kg}) \times (1/4) = 12.5 \text{ mg/kg}$. As an additional example, doses of 0.02, 0.08, 0.8, 2, and 8 mg/kg in the mouse equate to effect doses of 1.667 micrograms/kg, 6.67 micrograms/kg, 66.7 micrograms/kg, 166.7 micrograms/kg, and 0.667 mg/kg, respectively, in the human.

[0767] Pharmaceutical compositions containing Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention may be administered orally, rectally, parenterally, subcutaneously, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), buccally, or as an oral or nasal spray (e.g., via inhalation of a vapor or powder). In one embodiment, "pharmaceutically acceptable carrier" means a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. In a specific embodiment, "pharmaceutically acceptable" means approved by a regulatory agency of the federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly humans. Nonlimiting examples of suitable pharmaceutical carriers according to this embodiment are provided in "Remington's Pharmaceutical Sciences" by E.W. Martin, and include sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can be employed as liquid carriers, particularly for injectable solutions. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[0768] The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

[0769] In a preferred embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV compositions of the invention (including polypeptides, polynucleotides, and antibodies, and agonists and/or antagonists thereof) are administered subcutaneously.

[0770] In another preferred embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV compositions of the invention (including polypeptides, polynucleotides, and antibodies, and agonists and/or antagonists thereof) are administered intravenously.

[0771] Neutrokin-alpha and/or Neutrokin-alphaSV compositions of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or mirocapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

[0772] Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., *Biopolymers* 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (R. Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and R. Langer, *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., *Id.*) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

[0773] In a preferred embodiment, Neutrokin-alpha and/or Neutrokin-alphaSV compositions of the invention are formulated in a biodegradable, polymeric drug delivery system, for example as described in U.S. Patent Nos. 4,938,763; 5,278,201; 5,278,202; 5,324,519; 5,340,849; and 5,487,897 and in International Publication Numbers WO01/35929, WO00/24374, and WO00/06117 which are hereby incorporated by reference in their entirety. In specific preferred embodiments the Neutrokin-alpha and/or Neutrokin-alphaSV compositions of the invention are formulated using the ATRIGEL® Biodegradable System of Atrix Laboratories, Inc. (Fort Collins, Colorado). In other specific embodiments, Neutrokin-alpha and/or Neutrokin-alphaSV compositions of the invention are formulated using the ProLease® sustained relase sytem available from Alkermes, Inc. (Cambridge, MA).

[0774] Examples of biodegradable polymers which can be used in the formulation of Neutrokin-alpha and/or Neutrokin-alphaSV compositions, include but are not limited to,

polylactides, polyglycolides, polycaprolactones, polyanhydrides, polyamides, polyurethanes, polyesteramides, polyorthoesters, polydioxanones, polyacetals, polyketals, polycarbonates, polyorthocarbonates, polyphosphazenes, polyhydroxybutyrates, polyhydroxyvalerates, polyalkylene oxalates, polyalkylene succinates, poly(malic acid), poly(amino acids), poly(methyl vinyl ether), poly(maleic anhydride), polyvinylpyrrolidone, polyethylene glycol, polyhydroxycellulose, chitin, chitosan, and copolymers, terpolymers, or combinations or mixtures of the above materials. The preferred polymers are those that have a lower degree of crystallization and are more hydrophobic. These polymers and copolymers are more soluble in the biocompatible solvents than the highly crystalline polymers such as polyglycolide and chitin which also have a high degree of hydrogen-bonding. Preferred materials with the desired solubility parameters are the polylactides, polycaprolactones, and copolymers of these with glycolide in which there are more amorphous regions to enhance solubility. In specific preferred embodiments, the biodegradable polymers which can be used in the formulation of Neutrokinne-alpha and/or Neutrokinne-alphaSV compositions are poly(lactide-co-glycolides). Polymer properties such as molecular weight, hydrophobicity, and lactide/glycolide ratio may be modified to obtain the desired drug Neutrokinne-alpha and/or Neutrokinne-alphaSV release profile (See, e.g., Ravivarapu et al., Journal of Pharmaceutical Sciences 89:732-741 (2000), which is hereby incorporated by reference in its entirety).

[0775] It is also preferred that the solvent for the biodegradable polymer be non-toxic, water miscible, and otherwise biocompatible. Examples of such solvents include, but are not limited to, N-methyl-2-pyrrolidone, 2-pyrrolidone, C2 to C6 alkanols, C1 to C15 alcohols, diols, triols, and tetraols such as ethanol, glycerine propylene glycol, butanol; C3 to C15 alkyl ketones such as acetone, diethyl ketone and methyl ethyl ketone; C3 to C15 esters such as methyl acetate, ethyl acetate, ethyl lactate; alkyl ketones such as methyl ethyl ketone, C1 to C15 amides such as dimethylformamide, dimethylacetamide and caprolactam; C3 to C20 ethers such as tetrahydrofuran, or solketal; tweens, triacetin, propylene carbonate, decylmethylsulfoxide, dimethyl sulfoxide, oleic acid, 1-dodecylazacycloheptan-2-one, Other preferred solvents are benzyl alcohol, benzyl benzoate, dipropylene glycol, tributyrin, ethyl oleate, glycerin, glycofural, isopropyl myristate, isopropyl palmitate, oleic acid, polyethylene glycol, propylene carbonate, and

triethyl citrate. The most preferred solvents are N-methyl-2-pyrrolidone, 2-pyrrolidone, dimethyl sulfoxide, triacetin, and propylene carbonate because of the solvating ability and their compatibility.

[0776] Additionally, formulations comprising Neutrokin-alpha and/or Neutrokin-alphaSV compositions and a biodegradable polymer may also include release-rate modification agents and/or pore-forming agents. Examples of release-rate modification agents include, but are not limited to, fatty acids, triglycerides, other like hydrophobic compounds, organic solvents, plasticizing compounds and hydrophilic compounds. Suitable release rate modification agents include, for example, esters of mono-, di-, and tricarboxylic acids, such as 2-ethoxyethyl acetate, methyl acetate, ethyl acetate, diethyl phthalate, dimethyl phthalate, dibutyl phthalate, dimethyl adipate, dimethyl succinate, dimethyl oxalate, dimethyl citrate, triethyl citrate, acetyl tributyl citrate, acetyl triethyl citrate, glycerol triacetate, di(n-butyl) sebacate, and the like; polyhydroxy alcohols, such as propylene glycol, polyethylene glycol, glycerin, sorbitol, and the like; fatty acids; triesters of glycerol, such as triglycerides, epoxidized soybean oil, and other epoxidized vegetable oils; sterols, such as cholesterol; alcohols, such as C_{sub}6 -C_{sub}12 alkanols, 2-ethoxyethanol, and the like. The release rate modification agent may be used singly or in combination with other such agents. Suitable combinations of release rate modification agents include, but are not limited to, glycerin/propylene glycol, sorbitol/glycerine, ethylene oxide/propylene oxide, butylene glycol/adipic acid, and the like. Preferred release rate modification agents include, but are not limited to, dimethyl citrate, triethyl citrate, ethyl heptanoate, glycerin, and hexanediol. Suitable pore-forming agents that may be used in the polymer composition include, but are not limited to, sugars such as sucrose and dextrose, salts such as sodium chloride and sodium carbonate, polymers such as hydroxylpropylcellulose, carboxymethylcellulose, polyethylene glycol, and polyvinylpyrrolidone. Solid crystals that will provide a defined pore size, such as salt or sugar, are preferred.

[0777] In specific preferred embodiments the Neutrokin-alpha and/or Neutrokin-alphaSV compositions of the invention are formulated using the BEMA™ BioErodible Mucoadhesive System, MCA™ MucoCutaneous Absorption System, SMP™ Solvent MicroParticle System, or BCP™ BioCompatible Polymer System of Atrix Laboratories, Inc. (Fort Collins, Colorado).

[0778] Sustained-release compositions also include liposomally entrapped compositions of the invention (*see generally*, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide may be prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. (USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide therapy.

[0779] In another embodiment sustained release compositions of the invention include crystal formulations known in the art.

[0780] In yet an additional embodiment, the compositions of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

[0781] Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

[0782] For parenteral administration, in one embodiment, the Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

[0783] Generally, the formulations are prepared by contacting the Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a

solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

[0784] The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, sucrose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; preservatives, such as cresol, phenol, chlorobutanol, benzyl alcohol and parabens, and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

[0785] The Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide is typically formulated in such vehicles at a concentration of about 0.001 mg/ml to 100 mg/ml, or 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml or 1-10 mg/ml, at a pH of about 3 to 10, or 3 to 8, more preferably 5-8, most preferably 6-7. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide salts.

[0786] Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0787] Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide solution, and the

resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide using bacteriostatic Water-for-Injection.

[0788] Alternatively, Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide is stored in single dose containers in lyophilized form. The infusion selection is reconstituted using a sterile carrier for injection.

[0789] A composition of the invention may comprise Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide that is radiolabeled, for example, with radioactive isotopes of iodine. Compositions comprising iodinated forms of Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptides or fragments or variants thereof, may also comprise radioprotectants and plasma expanders such as sodium ascorbate, gentran-40, and glycerol. In specific embodiments, compositions of the invention comprising iodinated forms of Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptides or fragments or variants are formulated in 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Genetran-40. The above described compositions may be used as pharmaceutical compositions.

[0790] In specific embodiments, a composition of the invention comprises, at least 1 mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition of the invention comprises, at least 2 mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition of the invention comprises, at least 3 mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition of the invention comprises, at least 4 mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In particular embodiments, a composition of the invention comprises, about 4.6mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v)

Gentran-40. The above described compositions may be used as pharmaceutical compositions.

[0791] In specific embodiments, a composition of the invention comprises, about between 0.1 mg/mL and 20mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition of the invention comprises, between 1mg/mL and 10mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition of the invention comprises, between 2mg/mL and 8mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. In specific embodiments, a composition of the invention comprises, between 3mg/mL and 6mg/mL of an iodinated form of amino acid residues 134-285 of SEQ ID NO:2, 10.0mM sodium citrate, 140.0 mM sodium chloride, 8.7mM HEPES, 4% (w/v) sodium ascorbate, 3.3% (w/v) Gentran-40. The above described compositions may be used as pharmaceutical compositions.

[0792] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally, associated with such container(s) is a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

[0793] The compositions of the invention may be administered alone or in combination with other adjuvants. Adjuvants that may be administered with the compositions of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, compositions of the invention are administered in combination with alum. In another specific embodiment, compositions of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the compositions of the invention include, but are not limited to, Monophosphoryl

lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the compositions of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis, and/or PNEUMOVAX-23™. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0794] In a specific embodiment, compositions of the invention (e.g., Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention, Neutrokin-alpha and/or Neutrokin-alphaSV fragments and variants, and anti-Neutrokin-alpha and/or anti-Neutrokin-alphaSV antibodies) may be administered to patients as vaccine adjuvants. In a further specific embodiment, compositions of the invention may be administered as vaccine adjuvants to patients suffering from an immune-deficiency. In a further specific embodiment, compositions of the invention may be administered as vaccine adjuvants to patients suffering from HIV.

[0795] In a specific embodiment, compositions of the invention may be used to increase or enhance antigen-specific antibody responses to standard and experimental vaccines. In a specific embodiment, compositions of the invention may be used to enhance seroconversion in patients treated with standard and experimental vaccines. In another specific embodiment, compositions of the invention may be used to increase the number of unique epitopes recognized by antibodies elicited by standard and experimental vaccination.

[0796] In another specific embodiment, compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated therewith. In one embodiment,

compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose any Gram positive bacterial infection and/or any disease, disorder, and/or condition associated therewith. In another embodiment, compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with one or more members of the genus *Enterococcus* and/or the genus *Streptococcus*. In another embodiment, compositions of the invention are used in any combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with one or more members of the Group B streptococci. In another embodiment, compositions of the invention are used in combination with PNEUMOVAX-23™ to treat, prevent, and/or diagnose infection and/or any disease, disorder, and/or condition associated with *Streptococcus pneumoniae*.

[0797] The compositions of the invention may be administered alone or in combination with other therapeutic agents, including but not limited to, chemotherapeutic agents, antibiotics, antivirals, steroid and non-steroidal anti-inflammatories, conventional immunotherapeutic agents and cytokines. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

[0798] In one embodiment, the compositions of the invention are administered in combination with other members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the compositions of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), AIM-II (International Publication No. WO 97/34911), APRIL (J. Exp. Med. 188(6):1185-1190), endokine-alpha (International Publication No. WO 98/07880), TR6 (International

Publication No. WO 98/30694), OPG, and neutrokinin-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12.

[0799] In another embodiment, the compositions of the invention are invention are administered in combination with Neutrokinin-alpha receptors and/or Neurtokine-alpha SV receptors (e.g., TACI and BCMA) In preferred in emodiments the Neutrokinin-alpha receptors and/or Neurtokine-alpha SV receptors are soluble. In other preferred embodiments the the Neutrokinin-alpha receptors and/or Neurtokine-alpha SV receptors are fused to the FC region of an immunoglobulon molecule (e.g, amino acid residues 1-154 of TACI (GenBank accesision number AAC51790), or 1-48 of BCMA (GenBank accession number NP_001183) fused to the Fc region of an IgG molecule.

[0800] In a preferred embodiment, the compositions of the invention are administered in combination with CD40 ligand (CD40L), a soluble form of CD40L (e.g., AVREND™), bioloigically active fragments, variants, or derivatives of CD40L, anti-CD40L antibodies (e.g., agonistic or antagonistic antibodies), and/or anti-CD40 antibodies (e.g, agonistic or antagonistic antibodies).

[0801] In an additional embodiment, the compositions of the invention are administered alone or in combination with an anti-angiogenic agent(s). Anti-angiogenic agents that may be administered with the compositions of the invention include, but are not limited to, Angiostatin (Entremed, Rockville, MD), Troponin-1 (Boston Life Sciences, Boston, MA), anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel (Taxol), Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, VEGI, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

[0802] Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal

species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

[0803] Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetone and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

[0804] Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetone. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

[0805] A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include, but are not limited to, platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al.,

Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4- chloroanthronilic acid disodium or "CCA"; (Takeuchi et al., Agents Actions 36:312-316, 1992); and metalloproteinase inhibitors such as BB94.

[0806] Additional anti-angiogenic factors that may also be utilized within the context of the present invention include Thalidomide, (Celgene, Warren, NJ); Angiostatic steroid; AGM-1470 (H. Brem and J. Folkman *J Pediatr. Surg.* 28:445-51 (1993)); an integrin alpha v beta 3 antagonist (C. Storgard et al., *J Clin. Invest.* 103:47-54 (1999)); carboxynaminolmidazole; Carboxyamidotriazole (CAI) (National Cancer Institute, Bethesda, MD); Conbretastatin A-4 (CA4P) (OXiGENE, Boston, MA); Squalamine (Magainin Pharmaceuticals, Plymouth Meeting, PA); TNP-470, (Tap Pharmaceuticals, Deerfield, IL); ZD-0101 AstraZeneca (London, UK); APRA (CT2584); Benefin, Byrostatin-1 (SC339555); CGP-41251 (PKC 412); CM101; Dexrazoxane (ICRF187); DMXAA; Endostatin; Flavopridiol; Genestein; GTE; ImmTher; Iressa (ZD1839); Octreotide (Somatostatin); Panretin; Penacillamine; Photopoint; PI-88; Prinomastat (AG-3340) Purlytin; Suradista (FCE26644); Tamoxifen (Nolvadex); Tazarotene; Tetrathiomolybdate; Xeloda (Capecitabine); and 5-Fluorouracil.

[0807] Anti-angiogenic agents that may be administered in combination with the compositions of the invention may work through a variety of mechanisms including, but not limited to, inhibiting proteolysis of the extracellular matrix, blocking the function of endothelial cell-extracellular matrix adhesion molecules, by antagonizing the function of angiogenesis inducers such as growth factors, and inhibiting integrin receptors expressed on proliferating endothelial cells. Examples of anti-angiogenic inhibitors that interfere with extracellular matrix proteolysis and which may be administered in combination with the compositions of the invention include, but are not limited to, AG-3340 (Agouron, La Jolla, CA), BAY-12-9566 (Bayer, West Haven , CT), BMS-275291 (Bristol Myers Squibb, Princeton, NJ), CGS-27032A (Novartis, East Hanover, NJ), Marimastat (British Biotech, Oxford, UK), and Metastat (Aeterna, St-Foy, Quebec). Examples of anti-angiogenic inhibitors that act by blocking the function of endothelial cell-extracellular matrix adhesion molecules and which may be administered in combination with the

compositions of the invention include, but are not limited to, EMD-121974 (Merck KcgaA Darmstadt, Germany) and Vitaxin (Ixsys, La Jolla, CA/Medimmune, Gaithersburg, MD). Examples of anti-angiogenic agents that act by directly antagonizing or inhibiting angiogenesis inducers and which may be administered in combination with the compositions of the invention include, but are not limited to, Angiozyme (Ribozyme, Boulder, CO), Anti-VEGF antibody (Genentech, S. San Francisco, CA), PTK-787/ZK-225846 (Novartis, Basel, Switzerland), SU-101 (Sugen, S. San Francisco, CA), SU-5416 (Sugen/ Pharmacia Upjohn, Bridgewater, NJ), and SU-6668 (Sugen). Other anti-angiogenic agents act to indirectly inhibit angiogenesis. Examples of indirect inhibitors of angiogenesis which may be administered in combination with the compositions of the invention include, but are not limited to, IM-862 (Cytran, Kirkland, WA), Interferon-alpha, IL-12 (Roche, Nutley, NJ), and Pentosan polysulfate (Georgetown University, Washington, DC).

[0808] In particular embodiments, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of an autoimmune disease, such as for example, an autoimmune disease described herein.

[0809] In a particular embodiment, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of arthritis. In a more particular embodiment, the use compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of rheumatoid arthritis.

[0810] In particular embodiments, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of strokes.

[0811] In another embodiment, compositions of the invention are administered in combination with an anticoagulant. Anticoagulants that may be administered with the compositions of the invention include, but are not limited to, heparin, warfarin, and aspirin. In a specific embodiment, compositions of the invention are administered in combination with heparin and/or warfarin. In another specific embodiment, compositions of the invention are administered in combination with warfarin. In another specific embodiment, compositions of the invention are administered in combination with warfarin

and aspirin. In another specific embodiment, compositions of the invention are administered in combination with heparin. In another specific embodiment, compositions of the invention are administered in combination with heparin and aspirin.

[0812] In another embodiment, compositions of the invention are administered in combination with an agent that suppresses the production of anticardiolipin antibodies. In specific embodiments, the polynucleotides of the invention are administered in combination with an agent that blocks and/or reduces the ability of anticardiolipin antibodies to bind phospholipid-binding plasma protein beta 2-glycoprotein I (b2GPI).

[0813] In certain embodiments, compositions of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), DESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with compositions of the invention to treat, prevent, and/or diagnose AIDS and/or to treat, prevent, and/or diagnose HIV infection.

[0814] In certain embodiments, compositions of the invention are administered in combination with antiretroviral agents, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and/or protease inhibitors (PIs). NRTIs that may be administered in combination with the compositions of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™

(stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). NNRTIs that may be administered in combination with the compositions of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), REScriptor™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the compositions of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with compositions of the invention to treat AIDS and/or to prevent or treat HIV infection.

[0815] Additional NRTIs include LODENOSINE™ (F-ddA; an acid-stable adenosine NRTI; Triangle/Abbott; COVIRACIL™ (emtricitabine/FTC; structurally related to lamivudine (3TC) but with 3- to 10-fold greater activity *in vitro*; Triangle/Abbott); dOTC (BCH-10652, also structurally related to lamivudine but retains activity against a substantial proportion of lamivudine-resistant isolates; Biochem Pharma); Adefovir (refused approval for anti-HIV therapy by FDA; Gilead Sciences); PREVEON® (Adefovir Dipivoxil, the active prodrug of adefovir; its active form is PMEA-pp); TENOFOVIR™ (bis-POC PMPA, a PMPA prodrug; Gilead); DAPD/DXG (active metabolite of DAPD; Triangle/Abbott); D-D4FC (related to 3TC, with activity against AZT/3TC-resistant virus); GW420867X (Glaxo Wellcome); ZIAGEN™ (abacavir/159U89; Glaxo Wellcome Inc.); CS-87 (3'azido-2',3'-dideoxyuridine; WO 99/66936); and S-acyl-2-thioethyl (SATE)-bearing prodrug forms of β -L-FD4C and β -L-FddC (WO 98/17281).

[0816] Additional NNRTIs include COACTINON™ (Emivirine/MKC-442, potent NNRTI of the HEPT class; Triangle/Abbott); CAPRAVIRINE™ (AG-1549/S-1153, a next generation NNRTI with activity against viruses containing the K103N mutation; Agouron); PNU-142721 (has 20- to 50-fold greater activity than its predecessor delavirdine and is active against K103N mutants; Pharmacia & Upjohn); DPC-961 and DPC-963 (second-generation derivatives of efavirenz, designed to be active against viruses with the K103N mutation; DuPont); GW-420867X (has 25-fold greater activity than HBY097 and is active against K103N mutants; Glaxo Wellcome); CALANOLIDE A

(naturally occurring agent from the latex tree; active against viruses containing either or both the Y181C and K103N mutations); and Propolis (WO 99/49830).

[0817] Additional protease inhibitors include LOPINAVIR™ (ABT378/r; Abbott Laboratories); BMS-232632 (an azapeptide; Bristol-Myers Squibb); TIPRANAVIR™ (PNU-140690, a non-peptic dihydropyrrone; Pharmacia & Upjohn); PD-178390 (a nonpeptidic dihydropyrrone; Parke-Davis); BMS 232632 (an azapeptide; Bristol-Myers Squibb); L-756,423 (an indinavir analog; Merck); DMP-450 (a cyclic urea compound; Avid & DuPont); AG-1776 (a peptidomimetic with *in vitro* activity against protease inhibitor-resistant viruses; Agouron); VX-175/GW-433908 (phosphate prodrug of amprenavir; Vertex & Glaxo Welcome); CGP61755 (Ciba); and AGENERASE™ (amprenavir; Glaxo Wellcome Inc.).

[0818] Additional antiretroviral agents include fusion inhibitors/gp41 binders. Fusion inhibitors/gp41 binders include T-20 (a peptide from residues 643-678 of the HIV gp41 transmembrane protein ectodomain which binds to gp41 in its resting state and prevents transformation to the fusogenic state; Trimeris) and T-1249 (a second-generation fusion inhibitor; Trimeris).

[0819] Additional antiretroviral agents include fusion inhibitors/chemokine receptor antagonists. Fusion inhibitors/chemokine receptor antagonists include CXCR4 antagonists such as AMD 3100 (a bicyclam), SDF-1 and its analogs, and ALX40-4C (a cationic peptide), T22 (an 18 amino acid peptide; Trimeris) and the T22 analogs T134 and T140; CCR5 antagonists such as RANTES (9-68), AOP-RANTES, NNY-RANTES, and TAK-779; and CCR5/CXCR4 antagonists such as NSC 651016 (a distamycin analog). Also included are CCR2B, CCR3, and CCR6 antagonists. Chemokine receptor agonists such as RANTES, SDF-1, MIP-1 α , MIP-1 β , etc., may also inhibit fusion.

[0820] Additional antiretroviral agents include integrase inhibitors. Integrase inhibitors include dicafeoylquinic (DFQA) acids; L-chicoric acid (a dicafeoyltartaric (DCTA) acid); quinalizarin (QLC) and related anthraquinones; ZINTEVIR™ (AR 177, an oligonucleotide that probably acts at cell surface rather than being a true integrase inhibitor; Arondex); and naphthols such as those disclosed in WO 98/50347.

[0821] Additional antiretroviral agents include hydroxyurea-like compounds such as BCX-34 (a purine nucleoside phosphorylase inhibitor; Biocryst); ribonucleotide reductase inhibitors such as DIDOX™ (Molecules for Health); inosine monophosphate

dehydrogenase (IMPDH) inhibitors sucha as VX-497 (Vertex); and myvopholic acids such as CellCept (mycophenolate mofetil; Roche).

[0822] Additional antiretroviral agents include inhibitors of viral integrase, inhibitors of viral genome nuclear translocation such as arylene bis(methylketone) compounds; inhibitors of HIV entry such as AOP-RANTES, NNY-RANTES, RANTES-IgG fusion protein, soluble complexes of RANTES and glycosaminoglycans (GAG), and AMD-3100; nucleocapsid zinc finger inhibitors such as dithiane compounds; targets of HIV Tat and Rev; and pharmacoenhancers such as ABT-378.

[0823] Other antiretroviral therapies and adjunct therapies include cytokines and lymphokines such as MIP-1 α , MIP-1 β , SDF-1 α , IL-2, PROLEUKINTM (aldesleukin/L2-7001; Chiron), IL-4, IL-10, IL-12, and IL-13; interferons such as IFN- α 2a; antagonists of TNFs, NF κ B, GM-CSF, M-CSF, and IL-10; agents that modulate immune activation such as cyclosporin and prednisone; vaccines such as RemuneTM (HIV Immunogen), APL 400-003 (Apollon), recombinant gp120 and fragments, bivalent (B/E) recombinant envelope glycoprotein, rgp120CM235, MN rgp120, SF-2 rgp120, gp120/soluble CD4 complex, Delta JR-FL protein, branched synthetic peptide derived from discontinuous gp120 C3/C4 domain, fusion-competent immunogens, and Gag, Pol, Nef, and Tat vaccines; gene-based therapies such as genetic suppressor elements (GSEs; WO 98/54366), and intrakines (genetically modified CC chemokines targetted to the ER to block surface expression of newly synthesized CCR5 (Yang *et al.*, *PNAS* 94:11567-72 (1997); Chen *et al.*, *Nat. Med.* 3:1110-16 (1997)); antibodies such as the anti-CXCR4 antibody 12G5, the anti-CCR5 antibodies 2D7, 5C7, PA8, PA9, PA10, PA11, PA12, and PA14, the anti-CD4 antibodies Q4120 and RPA-T4, the anti-CCR3 antibody 7B11, the anti-gp120 antibodies 17b, 48d, 447-52D, 257-D, 268-D and 50.1, anti-Tat antibodies, anti-TNF- α antibodies, and monoclonal antibody 33A; aryl hydrocarbon (AH) receptor agonists and antagonists such as TCDD, 3,3',4,4',5-pentachlorobiphenyl, 3,3',4,4'-tetrachlorobiphenyl, and α -naphthoflavone (WO 98/30213); and antioxidants such as γ -L-glutamyl-L-cysteine ethyl ester (γ -GCE; WO 99/56764).

[0824] In other embodiments, compositions of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the compositions of the invention, include, but are

not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, compositions of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, compositions of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, compositions of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, compositions of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat, prevent, and/or diagnose an opportunistic cytomegalovirus infection. In another specific embodiment, compositions of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat, prevent, and/or diagnose an opportunistic fungal infection. In another specific embodiment, compositions of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat, prevent, and/or diagnose an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, compositions of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat, prevent, and/or diagnose an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, compositions of the invention are used in any combination with

LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat, prevent, and/or diagnose an opportunistic bacterial infection.

[0825] In a further embodiment, the compositions of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the compositions of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

[0826] In a further embodiment, the compositions of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the compositions of the invention include, but are not limited to, amoxicillin, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

[0827] Conventional nonspecific immunosuppressive agents, that may be administered in combination with the compositions of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs cyclophosphamide, cyclophosphamide IV, methylprednisolone, prednisolone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells. Other immunosuppressive agents, that may be administered in combination with the compositions of the invention include, but are not limited to, prednisolone, methotrexate, thalidomide, methoxsalen, rapamycin, leflunomide, mizoribine (BREDININT™), brequinar, deoxyspergualin, and azaspirane (SKF 105685).

[0828] In specific embodiments, compositions of the invention are administered in combination with immunosuppressants. Immunosuppressant preparations that may be administered with the compositions of the invention include, but are not limited to, ORTHOCLONE OKT® 3 (muromonab-CD3), SANDIMMUNE™, NEORAL™, SANGDYA™ (cyclosporine), PROGRAF® (FK506, tacrolimus), CELLCEPT® (mycophenolate mofetil, of which the active metabolite is mycophenolic acid), IMURAN™ (azathioprine), glucorticosteroids, adrenocortical steroids such as DELTASONE™ (prednisone) and HYDELTRASOL™ (prednisolone), FOLEX™ and MEXATE™ (methotrexate), OXSORALEN-ULTRA™ (methoxsalen) and RAPAMUNE™

(sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

[0829] In a preferred embodiment, the compositions of the invention are administered in combination with steroid therapy. Steroids that may be administered in combination with the compositions of the invention, include, but are not limited to, oral corticosteroids, prednisone, and methylprednisolone (e.g., IV methylprednisolone). In a specific embodiment, compositions of the invention are administered in combination with prednisone. In a further specific embodiment, the compositions of the invention are administered in combination with prednisone and an immunosuppressive agent. Immunosuppressive agents that may be administered with the compositions of the invention and prednisone are those described herein, and include, but are not limited to, azathioprine, cylophosphamide, and cyclophosphamide IV. In another specific embodiment, compositions of the invention are administered in combination with methylprednisolone. In a further specific embodiment, the compositions of the invention are administered in combination with methylprednisolone and an immunosuppressive agent. Immunosuppressive agents that may be administered with the compositions of the invention and methylprednisolone are those described herein, and include, but are not limited to, azathioprine, cylophosphamide, and cyclophosphamide IV.

[0830] In a preferred embodiment, the compositions of the invention are administered in combination with an antimalarial. Antimalarials that may be administered with the compositions of the invention include, but are not limited to, hydroxychloroquine, chloroquine, and/or quinacrine.

[0831] In a preferred embodiment, the compositions of the invention are administered in combination with an NSAID.

[0832] In a nonexclusive embodiment, the compositions of the invention are administered in combination with one, two, three, four, five, ten, or more of the following drugs: NRD-101 (Hoechst Marion Roussel), diclofenac (Dimethaid), oxaprozin potassium (Monsanto), mecasermin (Chiron), T-614 (Toyama), pemetrexed disodium (Eli Lilly), atreleuton (Abbott), valdecoxib (Monsanto), eltenac (Byk Gulden), campath, AGM-1470 (Takeda), CDP-571 (Celltech Chiroscience), CM-101 (CarboMed), ML-3000 (Merckle), CB-2431 (KS Biomedix), CBF-BS2 (KS Biomedix), IL-1Ra gene therapy (Valentis), JTE-522 (Japan Tobacco), paclitaxel (Angiotech), DW-166HC (Dong Wha), darbufelone

mesylate (Warner-Lambert), soluble TNF receptor 1 (synergen; Amgen), IPR-6001 (Institute for Pharmaceutical Research), trocade (Hoffman-La Roche), EF-5 (Scotia Pharmaceuticals), BIIL-284 (Boehringer Ingelheim), BIIF-1149 (Boehringer Ingelheim), LeukoVax (Inflammatics), MK-663 (Merck), ST-1482 (Sigma-Tau), and butixocort propionate (WarnerLambert).

[0833] In one embodiment, the compositions of the invention are administered in combination with one or more of the following drugs: infliximab (also known as Remicade™ Centocor, Inc.), Trocade (Roche, RO-32-3555), Leflunomide (also known as Arava™ from Hoechst Marion Roussel), Kineret™ (an IL-1 Receptor antagonist also known as Anakinra from Amgen, Inc.), SCIO-469 (p38 kinase inhibitor from Scios, Inc), and/or ASLERA™ (prasterone, dehydroepiandrosterone, GL701) from Genelabs Technologies Inc.

[0834] In a preferred embodiment, the compositions of the invention are administered in combination with one, two, three, four, five or more of the following drugs: methotrexate, sulfasalazine, sodium aurothiomalate, auranofin, cyclosporine, penicillamine, azathioprine, an antimalarial drug (e.g., as described herein), cyclophosphamide, chlorambucil, gold, ENBREL™ (Etanercept), anti-TNF antibody, LJP 394 (La Jolla Pharmaceutical Company, San Diego, California), and prednisolone.

[0835] In a more preferred embodiment, the compositions of the invention are administered in combination with an antimalarial, methotrexate, anti-TNF antibody, ENBREL™ and/or suflasalazine. In one embodiment, the compositions of the invention are administered in combination with methotrexate. In another embodiment, the compositions of the invention are administered in combination with anti-TNF antibody. In another embodiment, the compositions of the invention are administered in combination with methotrexate and anti-TNF antibody. In another embodiment, the compositions of the invention are administered in combination with suflasalazine. In another specific embodiment, the compositions of the invention are administered in combination with methotrexate, anti-TNF antibody, and suflasalazine. In another embodiment, the compositions of the invention are administered in combination ENBREL™. In another embodiment, the compositions of the invention are administered in combination with ENBREL™ and methotrexate. In another embodiment, the compositions of the invention are administered in combination with ENBREL™, methotrexate and suflasalazine. In

another embodiment, the compositions of the invention are administered in combination with ENBREL™, and sulfasalazine. In other embodiments, one or more antimalarials is combined with one of the above-recited combinations. In a specific embodiment, the compositions of the invention are administered in combination with an antimalarial (e.g., hydroxychloroquine), ENBREL™, methotrexate and sulfasalazine. In another specific embodiment, the compositions of the invention are administered in combination with an antimalarial (e.g., hydroxychloroquine), sulfasalazine, anti-TNF antibody, and methotrexate.

[0836] In an additional embodiment, compositions of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the compositions of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, compositions of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

[0837] In an additional embodiment, the compositions of the invention are administered alone or in combination with an anti-inflammatory agent. . Anti-inflammatory agents that may be administered with the compositions of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetidine, bendazac, benzylamine, bucolome, difenpiramide, ditazol, emorfazone, guiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

[0838] In specific embodiments, the compositions of the invention are administered alone or in combination with anti-CD4 antibody. In one embodiment, coadministration of the compositions of the invention with anti-CD4 antibody is envisioned for treatment of rheumatoid arthritis.

[0839] In specific embodiments, the compositions of the invention are administered alone or in combination with anti-IL-15 antibody. In one embodiment, coadministration

of the compositions of the invention with anti-IL-15 antibody is envisioned for treatment of rheumatoid arthritis.

[0840] In specific embodiments, the compositions of the invention are administered alone or in combination with CTLA4-Ig and LEA29Y. In one embodiment, coadministration of the compositions of the invention with CTLA4-Ig and LEA29Y is envisioned for treatment of rheumatoid arthritis.

[0841] In specific embodiments, the compositions of the invention are administered alone or in combination with anti-IL-6 Receptor antibody. In one embodiment, coadministration of the compositions of the invention with anti-IL-6 Receptor antibody is envisioned for treatment of rheumatoid arthritis.

[0842] In specific embodiments, the compositions of the invention are administered alone or in combination with anti-C5 (complement component) antibody. In one embodiment, coadministration of the compositions of the invention with anti-C5 antibody is envisioned for treatment of rheumatoid arthritis.

[0843] In specific embodiments, the compositions of the invention are administered alone or in combination with complement cascade inhibitors. Complement cascade inhibitors include, but are not limited to, anti-properdin antibodies (Gliatech); TP-10, a recombinant soluble type I complement receptor (AVANT Immunotheragenetics Inc.); Pexelizmab, a Complement C5 inhibitor (Alexion Pharmaceuticals Inc.); and 5G1.1, a monoclonal antibody that prevents cleavage of complement component C5 into its pro-inflammatory components. In one embodiment, coadministration of the compositions of the invention with complement cascade inhibitors are is envisioned for treatment of Inflammation, Rheumatoid arthritis, and/or cardiovascular disorders.

[0844] In another embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the compositions of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptapurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone,

estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephalen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dacarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

[0845] In a specific embodiment, compositions of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or combination of one or more of the components of CHOP. In one embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies, human monoclonal anti-CD20 antibodies. In another embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies and CHOP, or anti-CD20 antibodies and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, compositions of the invention are administered in combination with Rituximab. In a further embodiment, compositions of the invention are administered with Rituximab and CHOP, or Rituximab and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, compositions of the invention are administered in combination with tositumomab (anti-CD20 antibody from Coulter Pharmaceuticals, San Francisco, CA). In a further embodiment, compositions of the invention are administered with tositumomab and CHOP, or tositumomab and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. Tositumomab may optionally be associated with ¹³¹I. The anti-CD20 antibodies may optionally be associated with radioisotopes, toxins or cytotoxic prodrugs.

[0846] In another specific embodiment, the compositions of the invention are administered in combination Zevalin™. In a further embodiment, compositions of the invention are administered with Zevalin™ and CHOP, or Zevalin™ and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. Zevalin™ may be associated with one or more radisotopes. Particularly preferred isotopes are ⁹⁰Y and ¹¹¹In.

[0847] In an additional embodiment, the compositions of the invention are administered in combination with cytokines. Cytokines that may be administered with the compositions of the invention include, but are not limited to, GM-CSF, G-CSF, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-alpha, IFN-beta, IFN-gamma, TNF-alpha, and TNF-beta. In another embodiment, compositions of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, and IL-22. In preferred embodiments, the compositions of the invention are administered in combination with IL4 and IL10. Both IL4 and IL10 have been observed by the inventors to enhance Neutrokin-alpha mediated B cell proliferation.

[0848] In vitro, IFN gamma and IL-10 have each been observed by the inventors to enhance cell surface expression of Neutrokin-alpha in monocytes and macrophages (macrophages were obtained by culturing primary monocytes with 20ng/mL of M-CSF for 12-15 days), whereas IL-4 treatment decreased cell surface expression of Neutrokin-alpha in monocytes and macrophages. IL-4 administered with IL-10 resulted in a complete inhibition of the IL-10 induced cell surface expression of Neutrokin-alpha. IL-4 administered with IFN-gamma resulted in increased cell-surface expression of Neutrokin-alpha. Treatment of macrophages with IFN-gamma and IL-10 resulted in a 3 fold increase of soluble (active) Neutrokin-alpha released into the culture medium compared to untreated macrophages.

[0849] In an additional embodiment, the compositions of the invention are administered with a chemokine. In another embodiment, the compositions of the invention are administered with chemokine beta-8, chemokine beta-1, and/or macrophage inflammatory protein-4. In a preferred embodiment, the compositions of the invention are administered with chemokine beta-8.

[0850] In an additional embodiment, the compositions of the invention are administered in combination with an IL-4 antagonist. IL-4 antagonists that may be administered with the compositions of the invention include, but are not limited to: soluble IL-4 receptor polypeptides, multimeric forms of soluble IL-4 receptor polypeptides; anti-IL-4 receptor antibodies that bind the IL-4 receptor without transducing the biological signal elicited by IL-4, anti-IL4 antibodies that block binding of IL-4 to one or more IL-4

receptors, and muteins of IL-4 that bind IL-4 receptors but do not transduce the biological signal elicited by IL-4. Preferably, the antibodies employed according to this method are monoclonal antibodies (including antibody fragments, such as, for example, those described herein).

[0851] In an additional embodiment, the compositions of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the compositions of the invention include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

[0852] In an additional embodiment, the compositions of the invention are administered in combination with fibroblast growth factors. Fibroblast growth factors that may be administered with the compositions of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

[0853] Additionally, the compositions of the invention may be administered alone or in combination with other therapeutic regimens, including but not limited to, radiation therapy. Such combinatorial therapy may be administered sequentially and/or concomitantly.

Agonists and Antagonists - Assays and Molecules

[0854] The invention also provides a method of screening compounds to identify those which enhance or block the action of Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide on cells, such as its interaction with Neutrokinne-alpha and/or Neutrokinne-alphaSV binding molecules such as receptor molecules. An agonist is a compound which increases the natural biological functions of Neutrokinne-alpha and/or Neutrokinne-alphaSV or which functions in a manner similar to Neutrokinne-alpha and/or Neutrokinne-alphaSV while antagonists decrease or eliminate such functions.

[0855] In another embodiment, the invention provides a method for identifying a receptor protein or other ligand-binding protein which binds specifically to a Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide. For example, a cellular compartment, such as a membrane or a preparation thereof, may be prepared from a cell that expresses a molecule that binds Neutrokinne-alpha and/or Neutrokinne-alphaSV. The

preparation is incubated with labeled Neutrokin-alpha and/or Neutrokin-alphaSV and complexes of Neutrokin-alpha and/or Neutrokin-alphaSV bound to the receptor or other binding protein are isolated and characterized according to routine methods known in the art. Alternatively, the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide may be bound to a solid support so that binding molecules solubilized from cells are bound to the column and then eluted and characterized according to routine methods.

[0856] In the assay of the invention for agonists or antagonists, a cellular compartment, such as a membrane or a preparation thereof, may be prepared from a cell that expresses a molecule that binds Neutrokin-alpha and/or Neutrokin-alphaSV such as a molecule of a signaling or regulatory pathway modulated by Neutrokin-alpha and/or Neutrokin-alphaSV. The preparation is incubated with labeled Neutrokin-alpha and/or Neutrokin-alphaSV in the absence or the presence of a candidate molecule which may be a Neutrokin-alpha and/or Neutrokin-alphaSV agonist or antagonist. The ability of the candidate molecule to bind the binding molecule is reflected in decreased binding of the labeled ligand. Molecules which bind gratuitously, i.e., without inducing the effects of Neutrokin-alpha on binding the Neutrokin-alpha and/or Neutrokin-alphaSV binding molecule, are most likely to be good antagonists. Molecules that bind well and elicit effects that are the same as or closely related to Neutrokin-alpha and/or Neutrokin-alphaSV are agonists.

[0857] Neutrokin-alpha- and/or Neutrokin-alphaSV- like effects of potential agonists and antagonists may be measured, for instance, by determining activity of a second messenger system following interaction of the candidate molecule with a cell or appropriate cell preparation, and comparing the effect with that of Neutrokin-alpha and/or Neutrokin-alphaSV or molecules that elicit the same effects as Neutrokin-alpha and/or Neutrokin-alphaSV. Second messenger systems that may be useful in this regard include but are not limited to AMP guanylate cyclase, ion channel or phosphoinositide hydrolysis second messenger systems.

[0858] Another example of an assay for Neutrokin-alpha and/or Neutrokin-alphaSV antagonists is a competitive assay that combines Neutrokin-alpha and/or Neutrokin-alphaSV and a potential antagonist with membrane-bound receptor molecules or recombinant Neutrokin-alpha and/or Neutrokin-alphaSV receptor molecules under appropriate conditions for a competitive inhibition assay. Neutrokin-alpha and/or

Neutrokine-alphaSV can be labeled, such as by radioactivity, such that the number of Neutrokine-alpha and/or Neutrokine-alphaSV molecules bound to a receptor molecule can be determined accurately to assess the effectiveness of the potential antagonist.

[0859] Potential antagonists include small organic molecules, peptides, polypeptides (e.g., IL-13), and antibodies that bind to a polypeptide of the invention and thereby inhibit or extinguish its activity. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a receptor molecule, without inducing Neutrokine-alpha and/or Neutrokine-alphaSV induced activities, thereby preventing the action of Neutrokine-alpha and/or Neutrokine-alphaSV by excluding Neutrokine-alpha and/or Neutrokine-alphaSV from binding.

[0860] Other potential antagonists include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or RNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, J., Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA. For example, the 5' coding portion of a polynucleotide that encodes the extracellular domain of the polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of Neutrokine-alpha and/or Neutrokine-alphaSV. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into Neutrokine-alpha and/or Neutrokine-alphaSV polypeptide. The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may

be expressed in vivo to inhibit production of Neutrokinne-alpha and/or Neutrokinne-alphaSV.

[0861] In one embodiment, the Neutrokinne-alpha and/or Neutrokinne-alphaSV antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the Neutrokinne-alpha and/or Neutrokinne-alphaSV antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding Neutrokinne-alpha and/or Neutrokinne-alphaSV, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature* 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell* 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster, et al., *Nature* 296:39-42 (1982)), etc.

[0862] The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a Neutrokinne-alpha and/or Neutrokinne-alphaSV gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded Neutrokinne-alpha and/or Neutrokinne-alphaSV antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a Neutrokinne-alpha and/or Neutrokinne-alphaSV RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable

degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

[0863] Oligonucleotides that are complementary to the 5' end of the message, *e.g.*, the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, *Nature* 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non- translated, non-coding regions of Neutrokin-alpha and Neutrokin-alphaSV shown in Figures 1A-B and 5A-B, respectively, could be used in an antisense approach to inhibit translation of endogenous Neutrokin-alpha and/or Neutrokin-alphaSV mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'-, 3'- or coding region of Neutrokin-alpha and/or Neutrokin-alphaSV mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

[0864] The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger et al., 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre et al., *Proc. Natl. Acad. Sci.* 84:648-652 (1987); PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, *e.g.*, PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, *e.g.*, Krol et al., *BioTechniques* 6:958-976 (1988)) or intercalating agents. (See, *e.g.*, Zon, *Pharm. Res.* 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to

another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

[0865] The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiacytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

[0866] The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

[0867] In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

[0868] In yet another embodiment, the antisense oligonucleotide is an alpha-anomeric oligonucleotide. An alpha-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual beta-units, the strands run parallel to each other (Gautier et al., Nucl. Acids Res. 15:6625-6641 (1987)). The oligonucleotide is a 2-O-methylribonucleotide (Inoue et al., Nucl. Acids Res. 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1997)).

[0869] Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially

available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res. 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451 (1988)), etc.

[0870] While antisense nucleotides complementary to the Neutrokine-alpha and/or Neutrokine-alphaSV coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred.

[0871] Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy Neutrokine-alpha and/or Neutrokine-alphaSV mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of Neutrokine-alpha and Neutrokine-alphaSV (Figures 1A-B and 5A-B, respectively). Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the Neutrokine-alpha and/or Neutrokine-alphaSV mRNA; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

[0872] As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express Neutrokine-alpha and/or Neutrokine-alphaSV *in vivo*. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous Neutrokine-alpha and/or Neutrokine-alphaSV messages and inhibit

translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

[0873] Endogenous gene expression can also be reduced by inactivating or "knocking out" the Neutrokin-alpha and/or Neutrokin-alphaSV gene and/or its promoter using targeted homologous recombination. (E.g., see Smithies et al., *Nature* 317:230-234 (1985); Thomas & Capecchi, *Cell* 51:503-512 (1987); Thompson et al., *Cell* 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (e.g., see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art. The contents of each of the documents recited in this paragraph is herein incorporated by reference in its entirety.

[0874] In other embodiments, antagonists according to the present invention include soluble forms of Neutrokin-alpha and/or Neutrokin-alphaSV (e.g., fragments of Neutrokin-alpha shown in Figures 1A-B that include the ligand binding domain, TNF conserved domain, and/or extracellular domain of Neutrokin-alpha and/or Neutrokin-alphaSV and fragments of Neutrokin-alphaSV shown in Figures 5A-B that include the ligand binding domain, TNF conserved domain, and/or extracellular domain of Neutrokin-alpha and/or Neutrokin-alphaSV). Such soluble forms of the Neutrokin-alpha and/or Neutrokin-alphaSV, which may be naturally occurring or synthetic, antagonize Neutrokin-alpha and/or Neutrokin-alphaSV mediated signaling by competing with native Neutrokin-alpha and/or Neutrokin-alphaSV for binding to

Neutrokin-alpha and/or Neutrokin-alphaSV receptors (e.g., DR5 (See, International Publication No. WO 98/41629), TR10 (See, International Publication No. WO 98/54202), 312C2 (See, International Publication No. WO 98/06842), and TR11, TR11SV1, and TR11SV2 (See, U.S. Application Serial No. 09/176,200)), and/or by forming a multimer that may or may not be capable of binding the receptor, but which is incapable of inducing signal transduction. Preferably, these antagonists inhibit Neutrokin-alpha and/or Neutrokin-alphaSV mediated stimulation of lymphocyte (e.g., B-cell) proliferation, differentiation, and/or activation. Antagonists of the present invention also include antibodies specific for TNF-family ligands (e.g., CD30) and Neutrokin-alpha-Fc and/or Neutrokin-alphaSV-Fc fusion proteins.

[0875] By a "TNF-family ligand" is intended naturally occurring, recombinant, and synthetic ligands that are capable of binding to a member of the TNF receptor family and inducing and/or blocking the ligand/receptor signaling pathway. Members of the TNF ligand family include, but are not limited to, TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), FasL, CD40L, (TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), AIM-II (International Publication No. WO 97/34911), APRIL (J. Exp. Med. 188(6):1185-1190), endokine-alpha (International Publication No. WO 98/07880), neutrokin-alpha (International Publication No. WO 98/18921), CD27L, CD30L, 4-1BBL, OX40L, CD27, CD30, 4-1BB, OX40, and nerve growth factor (NGF). In preferred embodiments, the Neutrokin-alpha and/or Neutrokin-alphaSV TNF-family ligands of the invention are DR5 (See, International Publication No. WO 98/41629), TR10 (See, International Publication No. WO 98/54202), 312C2 (See, International Publication No. WO 98/06842), and TR11, TR11SV1, and TR11SV2 (See, U.S. Application Serial No. 09/176,200).

[0876] Antagonists of the present invention also include antibodies specific for TNF-family receptors or the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention. Antibodies according to the present invention may be prepared by any of a variety of standard methods using Neutrokin-alpha and/or Neutrokin-alphaSV immunogens of the present invention. As indicated, such Neutrokin-alpha and/or Neutrokin-alphaSV immunogens include the complete Neutrokin-alpha and Neutrokin-alphaSV polypeptides depicted in Figures 1A-B (SEQ ID NO:2) and Figures

5A-B (SEQ ID NO:19), respectively, (which may or may not include the leader sequence) and Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide fragments comprising, for example, the ligand binding domain, TNF-conserved domain, extracellular domain, transmembrane domain, and/or intracellular domain, or any combination thereof.

[0877] Polyclonal and monoclonal antibody agonists or antagonists according to the present invention can be raised according to the methods disclosed in Tartaglia and Goeddel, *J. Biol. Chem.* 267(7):4304-4307(1992); Tartaglia et al., *Cell* 73:213-216 (1993)), and PCT Application WO 94/09137 and are preferably specific to (i.e., bind uniquely to polypeptides of the invention having the amino acid sequence of SEQ ID NO:2. The term "antibody" (Ab) or "monoclonal antibody" (mAb) as used herein is meant to include intact molecules as well as fragments thereof (such as, for example, Fab and F(ab') fragments) which are capable of binding an antigen. Fab, Fab' and F(ab') fragments lack the Fc fragment intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl et al., *J. Nucl. Med.*, 24:316-325 (1983)).

[0878] In a preferred method, antibodies according to the present invention are mAbs. Such mAbs can be prepared using hybridoma technology (Kohler and Millstein, *Nature* 256:495-497 (1975) and U.S. Patent No. 4,376,110; Harlow et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988; *Monoclonal Antibodies and Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, New York, NY, 1980; Campbell, "Monoclonal Antibody Technology," In: *Laboratory Techniques in Biochemistry and Molecular Biology*, Volume 13 (Burdon et al., eds.), Elsevier, Amsterdam (1984)).

[0879] Proteins and other compounds which bind the Neutrokin-alpha and/or Neutrokin-alphaSV domains are also candidate agonists and antagonists according to the present invention. Such binding compounds can be "captured" using the yeast two-hybrid system (Fields and Song, *Nature* 340:245-246 (1989)). A modified version of the yeast two- hybrid system has been described by Roger Brent and his colleagues (Gyuris, *Cell* 75:791-803 (1993); Zervos et al., *Cell* 72:223-232 (1993)). Preferably, the yeast two-hybrid system is used according to the present invention to capture compounds which bind to the ligand binding domain, extracellular, intracellular, transmembrane, and death

domain of the Neutrokin-alpha and/or Neutrokin-alphaSV. Such compounds are good candidate agonists and antagonists of the present invention.

[0880] For example, using the two-hybrid assay described above, the extracellular or intracellular domain of the Neutrokin-alpha and/or Neutrokin-alphaSV receptor, or a portion thereof, may be used to identify cellular proteins which interact with Neutrokin-alpha and/or Neutrokin-alphaSV the receptor *in vivo*. Such an assay may also be used to identify ligands with potential agonistic or antagonistic activity of Neutrokin-alpha and/or Neutrokin-alphaSV receptor function. This screening assay has previously been used to identify protein which interact with the cytoplasmic domain of the murine TNF-RII and led to the identification of two receptor associated proteins. Rothe et al., *Cell* 78:681 (1994). Such proteins and amino acid sequences which bind to the cytoplasmic domain of the Neutrokin-alpha and/or Neutrokin-alphaSV receptors are good candidate agonist and antagonist of the present invention.

[0881] Other screening techniques include the use of cells which express the polypeptide of the present invention (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation, for example, as described in *Science*, 246:181-296 (1989). In another example, potential agonists or antagonists may be contacted with a cell which expresses the polypeptide of the present invention and a second messenger response, e.g., signal transduction may be measured to determine whether the potential antagonist or agonist is effective.

[0882] Agonists according to the present invention include naturally occurring and synthetic compounds such as, for example, TNF family ligand peptide fragments, transforming growth factor, neurotransmitters (such as glutamate, dopamine, *N*-methyl-D-aspartate), tumor suppressors (p53), cytolytic T cells and antimetabolites. Preferred agonists include chemotherapeutic drugs such as, for example, cisplatin, doxorubicin, bleomycin, cytosine arabinoside, nitrogen mustard, methotrexate and vincristine. Others include ethanol and -amyloid peptide. (*Science* 267:1457-1458 (1995)).

[0883] Preferred agonists are fragments of Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention which stimulate lymphocyte (e.g., B cell) proliferation, differentiation and/or activation. Further preferred agonists include polyclonal and monoclonal antibodies raised against the Neutrokin-alpha and/or Neutrokin-alphaSV polypeptides of the invention, or a fragment thereof. Such agonist

antibodies raised against a TNF-family receptor are disclosed in Tartaglia et al., *Proc. Natl. Acad. Sci. USA* 88:9292-9296 (1991); and Tartaglia et al., *J. Biol. Chem.* 267:4304-4307(1992). See, also, PCT Application WO 94/09137.

[0884] In an additional embodiment, immunoregulatory molecules such as, for example, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha, may be used as agonists of Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptides of the invention which stimulate lymphocyte (e.g., B cell) proliferation, differentiation and/or activation. In a specific embodiment, IL4 and/or IL10 are used to enhance the Neutrokinne-alpha- and/or Neutrokinne-alphaSV-mediated proliferation of B cells.

[0885] In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

[0886] Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and

Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

[0887] When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

[0888] In yet another embodiment of the invention, the activity of Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide can be reduced using a "dominant negative." To this end, constructs which encode defective Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide, such as, for example, mutants lacking all or a portion of the TNF-conserved domain, can be used in gene therapy approaches to diminish the activity of Neutrokinne-alpha and/or Neutrokinne-alphaSV on appropriate target cells. For example, nucleotide sequences that direct host cell expression of Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide in which all or a portion of the TNF-conserved domain is altered or missing can be introduced into monocytic cells or other cells or tissues (either by in vivo or ex vivo gene therapy methods described herein or otherwise known in the art). Alternatively, targeted homologous recombination can be utilized to introduce such deletions or mutations into the subject's endogenous Neutrokinne-alpha and/or Neutrokinne-alphaSV gene in monocytes. The engineered cells will express non-functional Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptides (i.e., a ligand (e.g., multimer) that may be capable of binding, but which is incapable of inducing signal transduction).

Chromosome Assays

[0889] The nucleic acid molecules of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes

according to the present invention is an important first step in correlating those sequences with genes associated with disease.

[0890] In certain preferred embodiments in this regard, the cDNA and/or polynucleotides herein disclosed is used to clone genomic DNA of a Neutrokin-alpha and/or Neutrokin-alphaSV gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA then is used for *in situ* chromosome mapping using well known techniques for this purpose.

[0891] In addition, in some cases, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with probes from the cDNA as short as 50 or 60 bp. For a review of this technique, see Verma *et al.*, *Human Chromosomes: A Manual Of Basic Techniques*, Pergamon Press, New York (1988).

[0892] Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance In Man*, available on-line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

[0893] Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

[0894] With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be

one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

[0895] Utilizing the techniques described above, the chromosomal location of Neutrokin-alpha and Neutrokin-alphaSV was determined with high confidence using a combination of somatic cell hybrids and radiation hybrids to chromosome position 13q34.

Examples

[0896] Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting. Many of the following examples are set forth referring specifically to Neutrokin-alpha polynucleotides and polypeptides of the invention. Each example may also be practiced to generate and/or examine Neutrokin-alphaSV polynucleotides and/or polypeptides of the invention. One of ordinary skill in the art would easily be able to direct the following examples to Neutrokin-alphaSV.

*Example 1a: Expression and Purification of "His-tagged" Neutrokin-alpha in *E. coli**

[0897] The bacterial expression vector pQE9 (pD10) is used for bacterial expression in this example. (QIAGEN, Inc., *supra*). pQE9 encodes ampicillin antibiotic resistance ("Ampr") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., *supra*, and suitable single restriction enzyme cleavage sites. These elements are arranged such that an inserted DNA fragment encoding a polypeptide expresses that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the amino terminus of that polypeptide.

[0898] The DNA sequence encoding the desired portion of the Neutrokin-alpha protein comprising the extracellular domain sequence is amplified from the deposited cDNA clone using PCR oligonucleotide primers which anneal to the amino terminal sequences of the desired portion of the protein and to sequences in the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to

facilitate cloning in the pQE9 vector are added to the 5' and 3' primer sequences, respectively.

[0899] For cloning the extracellular domain of the protein, the 5' primer has the sequence 5'-GTG GGA TCC AGC CTC CGG GCA GAG CTG-3' (SEQ ID NO:10) containing the underlined *Bam* HI restriction site followed by 18 nucleotides of the amino terminal coding sequence of the extracellular domain of the sequence in Figures 1A and 1B. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a DNA segment encoding any desired portion of the complete Neutrokin a protein shorter or longer than the extracellular domain of the form. The 3' primer has the sequence 5'-GTG AAG CTT TTA CAG CAG TTT CAA TGC ACC-3' (SEQ ID NO:11) containing the underlined *Hind* III restriction site followed by two stop codons and 18 nucleotides complementary to the 3' end of the coding sequence of the DNA sequence in Figures 1A and 1B.

[0900] The amplified DNA fragment and the vector pQE9 are digested with *Bam* HI and *Hind* III and the digested DNAs are then ligated together. Insertion of the DNA into the restricted pQE9 vector places the protein coding region downstream from the IPTG-inducible promoter and in-frame with an initiating AUG and the six histidine codons.

[0901] The ligation mixture is transformed into competent *E. coli* cells using standard procedures such as those described in Sambrook et al., *Molecular Cloning: a Laboratory Manual, 2nd Ed.*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kan^r"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing protein, is available commercially from QIAGEN, Inc., *supra*. Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing. Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 µg/ml) and kanamycin (25 µg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:25 to

1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-beta-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

[0902] The cells are then stirred for 3-4 hours at 4° C in 6M guanidine-HCl, pH 8. The cell debris is removed by centrifugation, and the supernatant containing the is loaded on to a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist, 1995, QIAGEN, Inc., *supra*). Briefly the supernatant is loaded on to the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the Neutrokinne-alpha and/or Neutrokinne-alphaSV polypeptide is eluted with 6 M guanidine-HCl, pH 5.

[0903] The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins can be eluted by the addition of 250 mM immidazole. Immidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4° C or frozen at -80° C.

Example 1b: Expression and Purification of Neutrokinne-alpha in E. coli

[0904] The bacterial expression vector pQE60 is used for bacterial expression in this example. (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311). pQE60 encodes ampicillin antibiotic resistance ("Ampr") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., *supra*, and suitable single

restriction enzyme cleavage sites. These elements are arranged such that a DNA fragment encoding a polypeptide may be inserted in such a way as to produce that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of that polypeptide. However, in this example, the polypeptide coding sequence is inserted such that translation of the six His codons is prevented and, therefore, the polypeptide is produced with no 6 X His tag.

[0905] The DNA sequence encoding the desired portion of the protein comprising the extracellular domain sequence is amplified from the deposited cDNA clone using PCR oligonucleotide primers which anneal to the amino terminal sequences of the desired portion of the protein and to sequences in the deposited construct 3' to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pQE60 vector are added to the 5' and 3' sequences, respectively.

[0906] For cloning the extracellular domain of the protein, the 5' primer has the sequence 5'-GTG TCA TGA GCC TCC GGG CAG AGC TG-3' (SEQ ID NO:12) containing the underlined *Bsp* HI restriction site followed by 17 nucleotides of the amino terminal coding sequence of the extracellular domain of the sequence in Figures 1A and 1B. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a desired portion of the complete protein shorter or longer than the extracellular domain of the form. The 3' primer has the sequence 5'-GTG AAG CTT TTA TTA CAG CAG TTT CAA TGC ACC-3' (SEQ ID NO:13) containing the underlined *Hind* III restriction site followed by two stop codons and 18 nucleotides complementary to the 3' end of the coding sequence in the DNA sequence in Figures 1A and 1B.

[0907] The amplified DNA fragments and the vector pQE60 are digested with *Bsp* HI and *Hind* III and the digested DNAs are then ligated together. Insertion of the DNA into the restricted pQE60 vector places the protein coding region including its associated stop codon downstream from the IPTG-inducible promoter and in-frame with an initiating AUG. The associated stop codon prevents translation of the six histidine codons downstream of the insertion point.

[0908] The ligation mixture is transformed into competent *E. coli* cells using standard procedures such as those described in Sambrook et al., *Molecular Cloning: a Laboratory Manual*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989).

E. coli strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kanr"), is used in carrying out the illustrative example described herein. This strain, which is only one of many that are suitable for expressing protein, is available commercially from QIAGEN, Inc., *supra*. Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

[0909] One of ordinary skill in the art recognizes that any of a number of bacterial expression vectors may be useful in place of pQE9 and pQE60 in the expression protocols presented in this example. For example, the novel pHE4 series of bacterial expression vectors, in particular, the pHE4-5 vector may be used for bacterial expression in this example (ATCC Accession No. 209311; and variations thereof). The plasmid DNA designated pHE4-5/MPIFD23 in ATCC Deposit No. 209311 is vector plasmid DNA which contains an insert which encodes another ORF. The construct was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, on September 30, 1997. Using the *Nde* I and *Asp* 718 restriction sites flanking the irrelevant MPIF ORF insert, one of ordinary skill in the art could easily use current molecular biological techniques to replace the irrelevant ORF in the pHE4-5 vector with the Neutrokinne-alpha ORF of the present invention.

[0910] The pHE4-5 bacterial expression vector includes a neomycin phosphotransferase gene for selection, an *E. coli* origin of replication, a T5 phage promoter sequence, two *lac* operator sequences, a Shine-Delgarno sequence, and the lactose operon repressor gene (*lacIq*). These elements are arranged such that an inserted DNA fragment encoding a polypeptide expresses that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the amino terminus of that polypeptide. The promoter and operator sequences of the pHE4-5 vector were made synthetically. Synthetic production of nucleic acid sequences is well known in the art (CLONETECH 95/96 Catalog, pages 215-216, CLONETECH, 1020 East Meadow Circle, Palo Alto, CA 94303).

[0911] Clones containing the desired Neutrokinne-alpha constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 μ g/ml) and kanamycin (25 μ g/ml). The O/N culture is used to inoculate a large culture, at a dilution

of approximately 1:25 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. isopropyl-beta-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

[0912] The cells are then stirred for 3-4 hours at 4°C in 6M guanidine-HCl, pH 8. The cell debris is removed by centrifugation, and the supernatant containing the Neutrokin a is dialyzed against 50 mM Na-acetate buffer pH 6, supplemented with 200 mM NaCl. Alternatively, the protein can be successfully refolded by dialyzing it against 500 mM NaCl, 20% glycerol, 25 mM Tris/HCl pH 7.4, containing protease inhibitors. After renaturation the protein can be purified by ion exchange, hydrophobic interaction and size exclusion chromatography. Alternatively, an affinity chromatography step such as an antibody column can be used to obtain pure protein. The purified protein is stored at 4°C or frozen at -80°C.

[0913] In certain embodiments, it is preferred to generate expression constructs as detailed in this Example to mutate one or more of the three cysteine residues in the Neutrokin-alpha polypeptide sequence. The cysteine residues in the Neutrokin-alpha polypeptide sequence are located at positions 147, 232, and 245 as shown in SEQ ID NO:2 and at positions 213 and 226 of the Neutrokin-alpha polypeptide sequence as shown in SEQ ID NO:19 (there is no cysteine in the Neutrokin-alphaSV polypeptide sequence which corresponds to Cys-147 in the Neutrokin-alpha polypeptide sequence because amino acid residues 143-160 of the Neutrokin-alpha polypeptide sequence are not present in the Neutrokin-alphaSV polypeptide sequence).

Example 2: Cloning, Expression, and Purification of Neutrokin-alpha Protein in a Baculovirus Expression System

[0914] In this illustrative example, the plasmid shuttle vector pA2GP is used to insert the cloned DNA encoding the extracellular domain of the protein, lacking its naturally associated intracellular and transmembrane sequences, into a baculovirus to express the extracellular domain of the Neutrokin-alpha protein, using a baculovirus leader and standard methods as described in Summers et al., *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station

Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by the secretory signal peptide (leader) of the baculovirus gp67 protein and convenient restriction sites such as *Bam* HI, *Xba* I and *Asp* 718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that expresses the cloned polynucleotide.

[0915] Many other baculovirus vectors could be used in place of the vector above, such as pAc373, pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

[0916] The cDNA sequence encoding an N-terminally deleted form of the extracellular domain of the Neutrokinin-alpha protein in the deposited clone, lacking the AUG initiation codon, the naturally associated intracellular and transmembrane domain sequences, and amino acids Gln-73 through Leu-79 shown in Figures 1A and 1B (SEQ ID NO:2), is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5'-GTG GGA TCC CCG GGC AGA GCT GCA GGG C-3' (SEQ ID NO:14) containing the underlined *Bam* HI restriction enzyme site followed by 18 nucleotides of the sequence of the extracellular domain of the Neutrokinin-alpha protein shown in Figures 1A and 1B, beginning with the indicated N-terminus of the extracellular domain of the protein. The 3' primer has the sequence 5'-GTG GGA TCC TTA TTA CAG CAG TTT CAA TGC ACC-3' (SEQ ID NO:15) containing the underlined *Bam* HI restriction site followed by two stop codons and 18 nucleotides complementary to the 3' coding sequence in Figures 1A and 1B.

[0917] In certain other embodiments, constructs designed to express the entire predicted extracellular domain of the Neutrokinin-alpha (i.e., amino acid residues Gln-73 through Leu-285) are preferred. One of skill in the art would be able to use the

polynucleotide and polypeptide sequences provided as SEQ ID NO:1 and SEQ ID NO:2, respectively, to design polynucleotide primers to generate such a clone.

[0918] In a further preferred embodiment, a pA2GP expression construct encodes amino acid residues Leu-112 through Leu-285 of the Neutrokinne-alpha polypeptide sequence shown as SEQ ID NO:2.

[0919] In another preferred embodiment, a pA2GP expression construct encodes amino acid residues Ser-78 through Leu-285 of the Neutrokinne-alpha polypeptide sequence shown as SEQ ID NO:2.

[0920] The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with *Bam* HI and again is purified on a 1% agarose gel. This fragment is designated herein F1.

[0921] The plasmid is digested with the restriction enzymes *Bam* HI and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). This vector DNA is designated herein "V1".

[0922] Fragment F1 and the dephosphorylated plasmid V1 are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Statagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria are identified that contain the plasmid with the human gene by digesting DNA from individual colonies using *Bam* HI and then analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing. This plasmid is designated herein pA2GP-Neutrokinne-alpha.

[0923] Five micrograms of the plasmid pA2GP-Neutrokinne-alpha is co-transfected with 1.0 microgram of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., *Proc. Natl. Acad. Sci. USA* 84: 7413-7417 (1987). One μ g of BaculoGold™ virus DNA and 5 micrograms of the plasmid pA2GP Neutrokinne-alpha are mixed in a sterile well of a microtiter plate containing 50 microliters of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards,

10 microliters Lipofectin plus 90 microliters Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27°C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27°C for four days.

[0924] After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Rockville, Maryland) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Rockville, MD, page 9-10). After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 microliters of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C. The recombinant virus is called V-Neutrokinin-alpha.

[0925] To verify the expression of the Neutrokinin-alpha gene Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus V-Neutrokinin-alpha at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 microcuries of ³⁵S-methionine and 5 microcuries ³⁵S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

[0926] Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the extracellular domain of the protein and thus the cleavage point and length of the secretory signal peptide.

[0927] In a specific experimental example, recombinant Neutrokine-alpha was purified from baculovirus infected Sf9 cell supernatants as follows. The insect cells were grown in EXCEL401 medium (JRH Scientific) with 1 % (v/v) fetal bovine serum. At 92 hours post-infection, the harvested supernatant was clarified by centrifugation at 18,000 x g followed by 0.45 m depth filtration. A de-lipid filtration step might be also used to remove the lipid contaminants and in turn to improve initial capturing of the Neutrokine-alpha protein.

[0928] The supernatant was loaded on to a set of Poros HS-50/HQ-50 in tandem mode. As alternatives, Toyopearl QAE, Toyopearl Super Q (Tosohass), Q-Sepharose (Pharmacia) and equivalent resins might be used. This step is used as a negative purification step to remove strong anion binding contaminants. The HS/HQ flow through material was adjusted to pH 7.5 with 1 M Tris-HCl pH 8, diluted with equal volume of 50 mM Tris-HCl pH 8, and loaded onto a poros PI-20 or PI-50 column. The PI column was washed first with 4 column volumes of 75 mM sodium chloride in 50 mM Tris-HCl at pH 7.5, then eluted using 3 to 5 column volumes of a stepwise gradient of 300 mM, 750 mM, 1500 mM sodium chloride in 50 mM Tris-HCl pH 7.5. Neutrokine-alpha protein appears as a 17 KD band on reduced SDS-PAGE and is present in the 0.75 M to 1.5M Sodium chloride fractions.

[0929] The PI fraction was further purified through a Sephadryl S100 HR (Pharmacia) size exclusion column equilibrated with 0.15 M sodium chloride, 50 mM sodium acetate at pH 6. The S200 fractions were mixed with sodium chloride to a final concentration of 3 M and loaded onto a Toyopearl Hexyl 650C (Tosohass) column. The Hexyl column was eluted with a linear gradient from 3 M to 0.05 M sodium chloride in 50 mM Sodium acetate pH 6 in 5 to 15 column volumes. The sodium chloride gradient can also be replaced by ammonium sulfate gradient of 1M to 0 M in 50 mM sodium acetate pH 6 in the Hexyl chromatographic step. Fractions containing purified Neutrokine-alpha as analyzed through SDS-PAGE were combined and dialyzed against a buffer containing 150 mM Sodium chloride, 50 mM Sodium acetate, pH 6.

[0930] The final purified Neutrokine-alpha protein expressed in a baculovirus system as explained herein has an N-terminus sequence which begins with amino acid residue Ala-134 of SEQ ID NO:2. RP-HPLC analysis shows a single peak of greater than 95% purity. Endotoxin level was below the detection limit in LAL assay.

[0931] In another example, recombinant Neutrokinne-alpha was purified from baculovirus infected Sf9 cell supernatants containing 0.25% bovine serum as follows.

[0932] The Sf9 supernatant was harvested by centrifugation at 18,000 x g. The supernatant was then treated with 10 mM calcium chloride in slightly alkaline conditions for 10-15 minutes followed by centrifugation and then 0.22 micrometer depth filtration. The resulting Sf-9 cell supernatant was then diluted 2-fold and loaded on to a Poros PI-50 column (available from PE Biosystems). The column was equilibrated with 50 mM Tris (pH=7.4). The PI-50 column was washed with 1 CV of 50 mM Tris (pH=7.4) and then eluted with 1.5 M NaCl in 50 mM NaOAc (pH=6) over 3 CV. The PI fraction was loaded on to a Sephadryl S200 column equilibrated with 50 mM NaOAc (pH=6), 125 mM NaCl. The S200 fraction was mixed with salts to final concentrations of 0.7 M ammonium sulfate and 0.6 M NaCl and loaded on to a Toyopearl Hexyl 650C column (available from Toso Haas) that had been equilibrated in a buffer containing 0.6 M NaCl, 0.7 M ammonium sulfate in 50 mM NaOAc (pH=6). The column was then washed with 2 CV of the same buffer. Recombinant Neutrokinne-alpha was then eluted stepwise with 3 CV of 50 mM NaOAc (pH=6) followed by 2 CV of 20% ethanol wash. The recombinant Neutrokinne-alpha protein was then eluted at the end of the ammonium sulfate (0.3 to 0 M salt) gradient. The appropriate fractions were pooled and dialyzed against a buffer containing 50 mM NaOAc (pH=6), and then passed through a Poros 50 HQ column. The HQ flow-through was diluted to 4 ms and loaded on to a Toyopearl DEAD 650M column and then eluted with 25 mM NaCitrate, 125 mM NaCl.

[0933] In another example, recombinant Neutrokinne-alpha was expressed and purified using a baculoviral vector system in Sf+ insect cells.

[0934] First, a polynucleotide encoding amino acid residues Ser-78 through Leu-285 of the Neutrokinne-alpha polypeptide sequence shown in Figures 1A and 1B (which is exactly identical to amino acid residues Ser-78 through Leu-285 of the Neutrokinne-alpha polypeptide sequence shown as SEQ ID NO:2) was subcloned into the baculovirus transfer construct PSC to generate a baculovirus expression plasmid. The pA2GP transfer vector, derived from pVL941, contains the gp67 signal peptide, a modified multiple cloning site, and the *lac Z* gene cloned downstream of the *Drosophila* heat-shock promoter for selection of blue plaques. Using the sequence of Neutrokinne-alpha (SEQ ID NO:2) and the sequence of the pA2GP vector, a cloning strategy was designed for

seamlessly fusing the PSC signal peptide coding sequence to the Neutrokin-alpha coding sequence at Ala-134 (SEQ ID NO:2 and Figures 1A and 1B) and inserting it into a PSC baculovirus transfer plasmid. The strategy involved the use of a two-stage polymerase chain reaction (PCR) procedure. First, primers were designed for amplifying the Neutrokin-alpha sequences. The 5' primer consisted of the sequence encoding Ala-134 and following residues (5'-GGT CGC CGT TTC TAA CGC GGC CGT TCA GGG TCC AGA AG-3'; SEQ ID NO:31), preceded by the sequence encoding the PSC signal peptide C-terminus. The 3' primer (5'-CTG GTT CGG CCC AAG GTA CCA AGC TTG TAC CTT AGA TCT TTT CTA GAT C-3'; SEQ ID NO:32) consisted of the reverse complement of the pA2GP vector sequence immediately downstream from the Neutrokin-alpha coding sequence, preceded by a *Kpn* I restriction endonuclease site and a spacer sequence (for increased cutting efficiency by *Kpn* I). PCR was performed with the pA2GP containing Neutrokin-alpha plasmid template and primers O-1887 and O-1888, and the resulting PCR product was purified using standard techniques.

[0935] An additional PCR reaction was performed using the PSC baculovirus transfer plasmid pMGS12 as a template. The pMGS12 plasmid consists of the AcNPV EcoRI "I" fragment inserted into pUC8, with the polyhedrin coding sequences after the ATG start codon replaced with the PSC signal peptide and a polylinker site. The PCR reaction used pMGS12 as a template, a 5' primer (5'-CTG GTA GTT CTT CGG AGT GTG-3'; SEQ ID NO:33) which annealed in AcNPV ORF603 upstream of the unique *Ngo*M IV and *Eco*R V sites, and a 3' primer (5'-CGC GTT AGA AAC GGC GAC C-3'; SEQ ID NO:34) which annealed to the 3' end of the sequence encoding the PSC signal peptide.

[0936] To generate a PCR product in which the PSC signal peptide was seamlessly fused to the Ala-134 of the Neutrokin-alpha coding sequence, the PCR product was combined with the PSC signal peptide-polyhedrin upstream region PCR product and subjected to an additional round of PCR. Because the 3' end of the PSC signal peptide PCR product (pMGS12 / O-959 / O-1044) overlapped the 5' end of the Neutrokin-alpha PCR product prepared with primers O-1887 / O-1888, the two PCR products were combined and overlap-extended by PCR using primers O-959 and O-1888.

[0937] The resulting overlap-extended PCR product containing the PSC signal peptide fused to the Neutrokin-alpha sequence subsequently was inserted into baculovirus transfer plasmid pMGS12. The PCR product was digested with *Ngo*M IV and *Kpn* I, and

the fragment was purified and ligated into *NgoM* IV-*Kpn* I-cut pMGS12. After transformation of competent *E. coli* DH5alpha cells with the ligation mix, colonies were picked and plasmid DNA mini-preps were prepared. Several positive clones from each ligation were identified by restriction digestion analysis of the plasmid DNA, and three clones (pAcC9669, pAcC9671, and pAcC9672) were selected for large scale plasmid purification. The resulting plasmid DNA was subjected to DNA sequence analysis to confirm and sequence the Neutrokinne-alpha insert.

[0938] The following steps describe the recovery and purification process of recombinant Neutrokinne-alpha from Sf+ insect cells. Unless stated otherwise, the process is conducted at 2-8°C.

Recovery

Step 1. CaCl₂ Treatment

[0939] Sf+ cell supernatant was harvested by centrifugation at 8,000 x g. Recovery buffer-1 (1M CaCl₂) was added to the supernatant so that the final concentration of CaCl₂ was 10 mM. (In a further preferred embodiment, 1M ZnCl₂ is used in place of 1M CaCl₂.) The pH of the solution was adjusted to 7.7 ± with Recovery buffer-2 (1M Tris pH 8 (± 0.2)). The solution was incubated for 15 minutes and then centrifuged at 8,000 x g.

Purification

Step 1. Chromatography on Poros PI-50 Column

[0940] Sf+ cell supernatant was loaded on to a Poros PI-50 column (PE Biosystem). The column was equilibrated in PI-1 buffer (50 mM Tris, 50 mM NaCl, pH 7.4 (± 0.2)). The PI-50 column was washed with 1-2 CV of PI-1 buffer and then eluted with PI-2 buffer (50 mM Na Citrate pH 6 (± 0.2)) over 3 CV linear gradient. The elution was monitored by ultraviolet (UV) absorbance at 280 nm. Fractions were collected across the eluate peak and analyzed by SDS page. Appropriate fractions were pooled.

Step 2. Chromatography on Toyopearl Hexyl 650C Column

[0941] The PI pool was mixed with salts to final concentrations of 0.7M (NH₄)₂SO₄ and loaded on to a Toyopearl Hexyl 650C (Toso Haas) column equilibrated in HIC-1 buffer (50 mM NaOAc, 0.6M NaCl, 0.7M (NH₄)₂SO₄ pH 6 (± 0.2)). The column was then

washed with 2 CV of HIC-1 buffer. Subsequently, recombinant Neutrokinne-alpha was then eluted stepwise with 3-5 CV of HIC-2 buffer (50mM NaOAc pH 6.0 (\pm 0.2)) followed by a 2 CV 20% ethanol wash. The elution was monitored by UV absorbance at 280 nm and conductivity. Fractions were collected across the eluate peak and analyzed by SDS-PAGE. The appropriate fractions were then pooled.

Step 3. Chromatography on SP sepharose FF

[0942] The Hexyl fraction was dialyzed and adjusted to pH 4.5 with SP-1 buffer (50 mM sodium acetate pH 4.5 (\pm 0.2)), diluted to 4 ms and loaded through a SP sepharose (cation exchanger, Pharmacia) column equilibrated with SP-1 buffer (50 mM sodium acetate pH 4.5 (\pm 0.2)). Recombinant Neutrokinne-alpha protein was then eluted from the SP column with SP-2 buffer (50 mM sodium acetate pH 5.5 (\pm 0.2)) at pH 5.5. The elution was then monitored by ultraviolet (UV) absorbance at 280 nm. Fractions were collected across the eluate peak and analyzed by SDS page. Appropriate fractions were pooled.

Step 4. Dialysis of Recombinant Neutrokinne-alpha

[0943] The SP fractions were placed into a 6-8 kd cutoff membrane device and then dialyzed or diafiltered into Dialysis Buffer (10 mM sodium citrate, 140 mM sodium chloride pH 6 (\pm 0.2)) overnight.

Step 5. Filtration and Fill

[0944] The protein concentration of the recombinant Neutrokinne-alpha solution from Step 6 was determined by bicinchoninic acid (BCA) protein assay. Recombinant Neutrokinne-alpha formulation was adjusted to the final protein concentration with the appropriate buffer and filtered under controlled conditions. The filtrate (bulk substance) was stored in suitable sterilized containers below -20°C.

[0945] In a specific embodiment, Neutrokinne-alpha protein of the invention produced as described infra was adjusted to a final protein concentration of 1 to 5 mg/ml and buffered in 10 mM sodium citrate, 140 mM sodium chloride, pH = 6.0 \pm (0.4) and stored at or below -20°C in Type 1 glass vials.

[0946] During chromatography runs, the processes are monitored by UV absorbance at 280 nm. When applicable, in-process chromatography intermediates are tested for conductivity, pH, and monitored by SDS and/or RP-HPLC.

[0947] Columns and purification equipment are cleaned and sanitized with 0.2 or 0.5 M NaOH followed by deionized water and then 0.1 or 0.5 M acetic acid. The column and purification equipment are rinsed with deionized water and, if necessary, stored in the appropriate storage solution. Prior to use, the equipment is equilibrated with appropriate buffers (as described herein or as is well known in the art).

[0948] In a further preferred embodiment, 1M ZnCl₂ is used in place of 1M CaCl₂ in Step 1 of the Recovery section described above. Also, in this embodiment, a combination of ZnCl₂ and CaCl₂ may be used. Many combinations of 0.1 M ZnCl₂ and 0.9 M CaCl₂, may be used in the Recovery process of recombinant Neutrokinne-alpha protein such as, for example, but not limited to, a combination of 0.1 M ZnCl₂ and 0.9 M CaCl₂, 0.2 M ZnCl₂ and 0.8 M CaCl₂, 0.3 M ZnCl₂ and 0.7 M CaCl₂, 0.4 M ZnCl₂ and 0.6 M CaCl₂, 0.5 M ZnCl₂ and 0.5 M CaCl₂, 0.6 M ZnCl₂ and 0.4 M CaCl₂, 0.7 M ZnCl₂ and 0.3 M CaCl₂, 0.8 M ZnCl₂ and 0.2 M CaCl₂, 0.9 M ZnCl₂ and 0.1 M CaCl₂, and others. However, the presence of EDTA will inhibit the recovery process. Moreover, the presence of ZnCl₂ and/or CaCl₂ in Recovery Buffer-1 will induce the formation of larger amounts of higher molecular weight (or molecular mass) Neutrokinne-alpha multimers.

Example 3: Cloning and Expression of Neutrokinne-alpha in Mammalian Cells

[0949] A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109).

Mammalian host cells that could be used include, human HeLa, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells, Chinese hamster ovary (CHO) cells CHO-K1, NSO and HEK 293 cells.

[0950] Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

[0951] The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., *Biochem J.* 227:277-279 (1991); Bebbington et al., *Bio/Technology* 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

[0952] The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., *Molecular and Cellular Biology*, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., *Cell* 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites *Bam* HI, *Xba* I and *Asp* 718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Example 3(a): Cloning and Expression in COS Cells

[0953] The expression plasmid, pNeutrokine-alpha-HA, is made by cloning a portion of the deposited cDNA encoding the extracellular domain of the protein into the expression vector pcDNAI/Amp or pcDNAIII (which can be obtained from Invitrogen, Inc.). To produce a soluble, secreted form of the polypeptide, the extracellular domain is fused to the secretory leader sequence of the human IL-6 gene.

[0954] The expression vector pcDNAI/amp contains: (1) an *E. coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an

SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron; (5) several codons encoding a hemagglutinin fragment (i.e., an "HA" tag to facilitate purification) followed by a termination codon and polyadenylation signal arranged so that a cDNA can be conveniently placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein described by Wilson et al., *Cell* 37: 767 (1984). The fusion of the HA tag to the target protein allows easy detection and recovery of the recombinant protein with an antibody that recognizes the HA epitope. pcDNAIII contains, in addition, the selectable neomycin marker.

[0955] A DNA fragment encoding the extracellular domain of the Neutrokin-alpha polypeptide is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The plasmid construction strategy is as follows. The Neutrokin-alpha cDNA of the deposited clone is amplified using primers that contain convenient restriction sites, much as described above for construction of vectors for expression of Neutrokin-alpha in *E. coli*. Suitable primers include the following, which are used in this example. The 5' primer, containing the underlined *Bam* HI site, a Kozak sequence, an AUG start codon, a sequence encoding the secretory leader peptide from the human IL-6 gene, and 18 nucleotides of the 5' coding region of the extracellular domain of Neutrokin-alpha protein, has the following sequence: 5'-GCG GGA TCC GCC ACC ATG AAC TCC TTC TCC ACA AGC GCC TTC GGT CCA GTT GCC TTC TCC CTG GGG CTG CTC CTG GTG TTG CCT GCT GCC TTC CCT GCC CCA GTT GTG AGA CAA GGG GAC CTG GCC AGC-3' (SEQ ID NO:16). The 3' primer, containing the underlined *Bam* HI restriction site and 18 of nucleotides complementary to the 3' coding sequence immediately before the stop codon, has the following sequence: 5'-GTG GGA TCC TTA CAG CAG TTT CAA TGC ACC-3' (SEQ ID NO:17).

[0956] The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with *Bam* HI and then ligated. The ligation mixture is transformed into *E. coli* strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is

isolated from resistant colonies and examined by restriction analysis or other means for the presence of the fragment encoding the Neutrokin-alpha extracellular domain.

[0957] For expression of recombinant Neutrokin-alpha, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook et al., *Molecular Cloning: a Laboratory Manual*, Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989). Cells are incubated under conditions for expression of Neutrokin-alpha by the vector.

[0958] Expression of the Neutrokin-alpha-HA fusion protein is detected by radiolabeling and immunoprecipitation, using methods described in, for example Harlow et al., *Antibodies: A Laboratory Manual, 2nd Ed.*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing 35 S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson et al. cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated proteins then are analyzed by SDS-PAGE and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

Example 3(b): Cloning and Expression in CHO Cells

[0959] The vector pC4 is used for the expression of Neutrokin-alpha protein. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). To produce a soluble, secreted form of the Neutrokin-alpha polypeptide, the portion of the deposited cDNA encoding the extracellular domain is fused to the secretory leader sequence of the human IL-6 gene. The vector plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., Alt, F. W., Kellems, R. M., Bertino, J. R., and Schimke, R. T., 1978, *J. Biol. Chem.* 253:1357-1370, Hamlin, J.

L. and Ma, C. 1990, *Biochem. et Biophys. Acta*, 1097:107-143, Page, M. J. and Sydenham, M. A. 1991, *Biotechnology* 9:64-68). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach may be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

[0960] Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rouse Sarcoma Virus (Cullen, et al., *Molecular and Cellular Biology*, March 1985:438-447) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart et al., *Cell* 41:521-530 (1985)). Downstream of the promoter are the following single restriction enzyme cleavage sites that allow the integration of the genes: BamHI, Xba I, and Asp718. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human beta-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLV. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the Neutrokine-alpha in a regulated way in mammalian cells (Gossen, M., & Bujard, H. 1992, *Proc. Natl. Acad. Sci. USA* 89: 5547-5551). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

[0961] The plasmid pC4 is digested with the restriction enzymes *Bam* HI and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

[0962] The DNA sequence encoding the extracellular domain of the Neutrokine-alpha protein is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer, containing the underlined *Bam* HI site, a Kozak

sequence, an AUG start codon, a sequence encoding the secretory leader peptide from the human IL-6 gene, and 18 nucleotides of the 5' coding region of the extracellular domain of Neutrokine-alpha protein, has the following sequence: 5'-GCG GGA TCC GCC ACC ATG AAC TCC TTC TCC ACA AGC GCC TTC GGT CCA GTT GCC TTC TCC CTG GGG CTG CTC CTG GTG TTG CCT GCT GCC TTC CCT GCC CCA GTT GTG AGA CAA GGG GAC CTG GCC AGC-3' (SEQ ID NO:16). The 3' primer, containing the underlined *Bam* HI and 18 of nucleotides complementary to the 3' coding sequence immediately before the stop codon, has the following sequence: 5'-GTG GGA TCC TTA CAG CAG TTT CAA TGC ACC-3' (SEQ ID NO:17).

[0963] The amplified fragment is digested with the endonuclease *Bam* HI and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

[0964] Chinese hamster ovary cells lacking an active DHFR gene are used for transfection. Five μ g of the expression plasmid pC4 is cotransfected with 0.5 μ g of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 μ M, 2 μ M, 5 μ M, 10 μ M, 20 μ M). The same procedure is repeated until clones are obtained which grow at a concentration of 100-200 μ M. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

[0965] At least six Neutrokine-alpha expression constructs have been generated by the inventors herein to facilitate the production of Neutrokine-alpha and/or

Neutrokin-alphaSV polypeptides of several sizes and in several systems. The expression constructs are as follows: (1) pNa.A71-L285 (expresses amino acid residues Ala-71 through Leu-285), (2) pNa.A81-L285 (expresses amino acid residues Ala-81 through Leu-285), (3) pNa.L112-L285 (expresses amino acid residues Leu-112 through Leu-285), (4) pNa.A134-L285 (expresses amino acid residues Ala-134 through Leu-285), (5) pNa.L147-L285 (expresses amino acid residues Leu-147 through Leu-285), and (6) pNa.G161-L285 (expresses amino acid residues Gly-161 through Leu-285).

[0966] In preferred embodiments, the expression constructs are used to express various Neutrokin-alpha muteins from bacterial, baculoviral, and mammalian systems.

[0967] In certain additional preferred embodiments, the constructs express a Neutrokin-alpha polypeptide fragment fused at the N- and/or C-terminus to a heterologous polypeptide, e.g., the signal peptide from human IL-6, the signal peptide from CK-beta8 (amino acids -21 to -1 of the CK-beta8 sequence disclosed in published PCT application PCT/US95/09058), or the human IgG Fc region. Other sequences could be used which are known to those of skill in the art.

Example 4: Tissue distribution of Neutrokin-alpha mRNA expression

[0968] Northern blot analysis is carried out to examine Neutrokin-alpha gene expression in human tissues, using methods described by, among others, Sambrook *et al.*, cited above. A cDNA probe containing the entire nucleotide sequence of the Neutrokin-alpha protein (SEQ ID NO:1) is labeled with ^{32}P using the *rediprime*TM DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using a CHROMA SPIN-100TM column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for Neutrokin-alpha and/or Neutrokin-alpha mRNA.

[0969] Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) are obtained from Clontech and are examined with the labeled probe using ExpressHybTM hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70° C overnight, and films developed according to standard procedures.

[0970] To determine the pattern of Neutrokin-alpha and/or Neutrokin-alpha expression a panel of multiple tissue Northern blots were probed. This revealed predominant expression of single 2.6 kb mRNA in peripheral blood leukocytes, spleen, lymph node and bone marrow, and detectable expression in placenta, heart, lung, fetal liver, thymus and pancreas. Analysis of a panel of cell lines demonstrated high expression of Neutrokin-alpha and/or Neutrokin-alpha in HL60 cells, detectable expression in K562, but no expression in Raji, HeLa, or MOLT-4 cells. Overall it appears that Neutrokin-alpha and/or Neutrokin-alpha mRNA expression is enriched in the immune system.

Example 5: Gene Therapy Using Endogenous Neutrokin-alpha Gene

[0971] Another method of gene therapy according to the present invention involves operably associating the endogenous Neutrokin-alpha sequence with a promoter via homologous recombination as described, for example, in U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired. Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous Neutrokin-alpha, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of Neutrokin-alpha so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

[0972] The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together

in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

[0973] In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

[0974] Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous Neutrokin-alpha sequence. This results in the expression of Neutrokin-alpha in the cell. Expression may be detected by immunological staining, or any other method known in the art.

[0975] Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na₂ HPO₄, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately 3X10⁶ cells/ml. Electroporation should be performed immediately following resuspension.

[0976] Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the Neutrokin-alpha locus, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3'end. Two Neutrokin-alpha non-coding sequences are amplified via PCR: one Neutrokin-alpha non-coding sequence (Neutrokin-alpha fragment 1) is amplified with a HindIII site at the 5' end and an XbaI site at the 3'end; the other Neutrokin-alpha non-coding sequence (Neutrokin-alpha fragment 2) is amplified with a BamHI site at the 5'end and a HindIII site at the 3'end. The CMV promoter and Neutrokin-alpha fragments are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; Neutrokin-alpha fragment 1 - XbaI; Neutrokin-alpha fragment 2 - BamHI) and ligated together. The resulting

ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

[0977] Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 μ g/ml. 0.5 ml of the cell suspension (containing approximately 1.5.X10⁶ cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 μ F and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

[0978] Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37°C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

[0979] The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

Example 6: Neutrokinne-alpha, a Novel Member of the Tumor Necrosis Factor Ligand Family that Functions as a B Lymphocyte Stimulator

[0980] A 285 amino acid protein was identified in a human neutrophil/monocyte-derived cDNA library that shared significant homology within its predicted extracellular receptor-ligand binding domain to APRIL (28.7%) (Hahne, M., et al., *J.Exp.Med.* 188,1185-90 (1998)), TNF-alpha (16.2%) (Pennica, D., et al., *Nature* 312,724-729 (1984)) and LT-alpha (14.1%) (Gray, *Nature* 312,721-724 (1984)) (Figures 7A-1 and 7A-2). We have designated this cytokine Neutrokinne-alpha (we have also designated this molecule as B Lymphocyte Stimulator (BLyS) based on its biological activity). Hydrophobicity analyses of the the Neutrokinne-alpha protein sequence have revealed a potential transmembrane spanning domain between amino acid residues 47 and 73 which

is preceded by non-hydrophobic amino acids suggesting that Neutrokinne-alpha, like other members of the TNF ligand family, is a type II membrane bound protein (Cosman, D. *Stem Cells.* 12:440-55 (1994)). Expression of this cDNA in mammalian cells (HEK 293 and Chinese Hamster Ovary) and Sf9 insect cells identified a 152 amino acid soluble form with an N-terminal sequence beginning with the alanine residue at amino acid 134 (arrow in Figures 7A-1 and 7A-2). Reconstruction of the mass to charge ratio defined a mass for Neutrokinne-alpha of 17,038 Daltons, a value in consistent with that predicted for this 152 amino acid protein with a single disulfide bond (17037.5 Daltons).

[0981] Using human/hamster somatic cell hybrids and a radiation-hybrid mapping panel, the gene encoding Neutrokinne-alpha was found linked to marker SHGC-36171 which maps to human chromosome 13q34, a region not previously associated with any other member of the TNF superfamily of genes (Cosman, D. *Stem Cells.* 12:440-55 (1994)).

[0982] The expression profile of Neutrokinne-alpha was assessed by Northern blot (Figure 7B) and flow cytometric analyses (Table V and Figures 8A, 8B and 8C). Neutrokinne-alpha is encoded by a single 2.6kb mRNA found at high levels in peripheral blood leukocytes, spleen, lymph node and bone marrow. Lower expression levels were detected in placenta, heart, lung, fetal liver, thymus and pancreas. Among a panel of cell lines, Neutrokinne-alpha mRNA was detected in HL-60 and K562, but not in Raji, HeLa, or MOLT-4 cells. These results were confirmed by flow cytometric analyses using the Neutrokinne-alpha-specific mAb 2E5. As shown in Table V, Neutrokinne-alpha expression is not detected on T or B lineage cells but rather restricted to cells within the myeloid origin. Further analyses of normal blood cell types demonstrated significant expression on resting monocytes that was upregulated approximately 4-fold following exposure of cells to IFN-gamma (100 U/mL) for three days (Figures 8A and 8B). A concomitant increase in Neutrokinne-alpha-specific mRNA was also detected (Figure 8C). By contrast, Neutrokinne-alpha was not expressed on freshly isolated peripheral blood granulocytes, T cells, B cells, or NK cells.

[0983] Purified recombinant Neutrokinne-alpha ("rNeutrokinne-alpha") was assessed for its ability to induce activation, proliferation, differentiation or death in numerous cell based assays involving B cells, T cells, monocytes, NK cells, hematopoietic progenitors, and a variety of cell types of endothelial and epithelial origin. Among these assays,

Neutrokin-alpha was specifically found to increase B cell proliferation in a standard co-stimulatory assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM as priming agents (Sieckmann, D.G., *et al.*, *J.Exp.Med.* 147:814-29 (1978); Ringden, O., *et al.*, *Scand.J.Immunol.* 6:1159-69 (1977)). As shown in Figure 9A, recombinant Neutrokin-alpha induced a dose-dependent proliferation of tonsillar B cells. This response was similar to that of rIL2 over the dose range from 0.1 to 10,000 ng/mL. Neutrokin-alpha also induces B cell proliferation when cultured with cells co-stimulated with immobilized anti-IgM (Figure 9B). A dose-dependent response is readily observed as the amount of crosslinking agent increases in the presence of a fixed concentration of either IL2 or rNeutrokin-alpha.

[0984] In an attempt to correlate the specific biological activity on B cells with receptor expression, purified Neutrokin-alpha was biotinylated. The resultant biotin-Neutrokin-alpha protein retained biological function in the standard B cell proliferation assays. Lineage-specific analyses of whole human peripheral blood cells indicated that binding of biotinylated Neutrokin-alpha was undetectable on T cells, monocytes, NK cells and granulocytes as assessed by CD3, CD14, CD56, and CD66b respectively (Figures 10A, 10B, 10C, 10D and 10E). In contrast, biotinylated Neutrokin-alpha bound peripheral CD20⁺ B cells. Receptor expression was also detected on the B cell tumor lines REH, ARH-77, Raji, Namalwa, RPMI 8226, and IM-9 but not any of the myeloid-derived lines tested including THP-1, HL-60, K-562, and U-937. Representative flow cytometric profiles for the myeloma cell line IM-9 and the histiocytic line U-937 are shown in Figures 10F and 10G. Similar results were also obtained using a biologically active FLAG-tagged Neutrokin-alpha protein instead of the chemically modified biotin-Neutrokin-alpha. Taken together, these results confirm that Neutrokin-alpha displays a clear B cell tropism in both its receptor distribution and biological activity. It remains to be shown whether cellular activation may induce expression of Neutrokin-alpha receptors on peripheral blood cells, other normal cell types or established cell lines.

[0985] To examine the species specificity of Neutrokin-alpha, mouse splenic B cells were cultured in the presence of human Neutrokin-alpha and SAC. Results demonstrate that rNeutrokin-alpha induced *in vitro* proliferation of murine splenic B cells and bound to a cell surface receptor on these cells. Interestingly, immature surface Ig negative B cell

precursors isolated from mouse bone marrow did not proliferate in response to Neutrokin-alpha nor did they bind the ligand.

[0986] To assess the *in vivo* activity of rNeutrokin-alpha, BALB/c mice (3/group) were injected (i.p.) twice per day with buffer only, or 0.08 mg/kg, 0.8 mg/kg, 2 mg/kg or 8 mg/kg of rNeutrokin-alpha. Mice received this treatment for 4 consecutive days at which time they were sacrificed and various tissues and serum collected for analyses. In an alternative embodiment, BALB/c mice may be injected (i.p.) twice per day with any amount of rNeutrokin-alpha in a range of 0.01 to 10 mg/kg. In a preferred embodiment, BALB/c mice are injected (i.p.) twice per day with any amount of rNeutrokin-alpha in a range of 0.01 to 3 mg/kg (specific preferred exemplary dosages in this embodiment include, but are not limited to, 0.01 mg/kg, 0.02 mg/kg, 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.06 mg/kg, 0.07 mg/kg, 0.08 mg/kg, 0.09 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1.0 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg, 1.6 mg/kg, 1.6 mg/kg, 1.7 mg/kg, 1.8 mg/kg, 1.9 mg/kg, 2.0 mg/kg, 2.1 mg/kg, 2.2 mg/kg, 2.3 mg/kg, 2.4 mg/kg, 2.5 mg/kg, 2.6 mg/kg, 2.7 mg/kg, 2.8 mg/kg, 2.9 mg/kg, and 3.0 mg/kg). In an additional preferred embodiment, BALB/c mice are injected (i.p.) twice per day with any amount of rNeutrokin-alpha in a range of 0.02 to 2 mg/kg (specific preferred exemplary dosages in this embodiment include, but are not limited to, 0.02 mg/kg, 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.06 mg/kg, 0.07 mg/kg, 0.08 mg/kg, 0.09 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1.0 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg, 1.6 mg/kg, 1.6 mg/kg, 1.7 mg/kg, 1.8 mg/kg, 1.9 mg/kg, and 2.0 mg/kg).

[0987] Microscopically, the effects of Neutrokin-alpha administration were clearly evident in sections of spleen stained with routine hematoxylin and eosin (H&E) and immunohistochemically with a mAb specific for CD45R(B220) (Figure 11A). Normal splenic architecture was altered by a dramatic expansion of the white pulp marginal zone and a distinct increase in cellularity of the red pulp (Figure 11A). Marginal zone expansion appeared to be the result of increased numbers of lymphocytes expressing the B cell marker CD45R(B220). In addition, the T cell dense periarteriolar lymphoid sheath (PALS) areas were also infiltrated by moderate numbers of CD45R(B220) positive cells. This suggests the white pulp changes were due to increased numbers of B cells. The

densely packed cell population that frequently filled red pulps spaces did not stain with CD45R(B220). Additional experiments will be required to characterize all the cell types involved and further define the mechanism by which Neutrokin-alpha alters splenic architecture.

[0988] Flow cytometric analyses of the spleens from mice treated with 2 mg/kg Neutrokin-alpha-treated indicated that Neutrokin-alpha increased the proportion of mature (CD45R(B220)^{dull}, ThB^{bright}) B cells approximately 10-fold over that observed in control mice (Figures 11B and 11C). Further analyses performed in which mice were treated with buffer, 0.08 mg/kg, 0.8 mg/kg, 2 mg/kg, or 8 mg/kg Neutrokin-alpha indicated that 0.08 mg/kg, 0.8 mg/kg, and 2 mg/kg each increased the proportion of mature (CD45R(B220)^{dull}, ThB^{bright}) B cells approximately 10-fold over that observed in control mice, whereas buffer and 8 mg/kg produced approximately equal proportions of mature B cells. *See, Table IV.*

Table IV. FACS Analysis of Mouse Spleen B cell Population.

<i>Neutrokin-alpha</i> (mg/kg)	<i>% Mature B Cells (R2)</i>	<i>% CD45R-positive (R1)</i>
Control (buffer)	1.26	52.17
0.08 mg/kg	16.15	56.53
0.8 mg/kg	18.54	57.56
2 mg/kg	16.54	57.55
8 mg/kg	1.24	61.42

[0989] A potential consequence of increased mature B cell representation *in vivo* is a relative increase in serum Ig titers. Accordingly, serum IgA, IgG and IgM levels were compared between buffer and Neutrokin-alpha-treated mice (Figures 11D, 11E, and 11F). Neutrokin-alpha administration resulted in a 2- and 5-fold increase in IgA and IgM serum levels respectively. Interestingly, circulating levels of IgG did not increase.

[0990] Moreover, a dose-dependent response was observed in serum IgA titers in mice treated with various amounts of Neutrokin-alpha over a period of four days, whereas no apparent dose-dependency was observed by administration of the same amounts of Neutrokin-alpha over a period of two days. In the case of administration over four days, administration of 8, 2, 0.8, 0.08, and 0 mg/kg Neutrokin-alpha resulted in serum IgA

titors of approximately 800 micrograms/ml, 700 micrograms/ml, 400 micrograms/ml, 200 micrograms/ml and 200 micrograms/ml. That is, administration of 8, 2, 0.8, and 0.08 mg/kg Neutrokin-alpha over four days resulted in approximately 4-fold, 3.75-fold, 2-fold, and minimal-fold, respectively, increases in IgA serum levels over background or basal levels observed by administration of buffer only. In an alternative embodiment, these experiments may be performed with any amount of rNeutrokin-alpha in a range of 0.01 to 10 mg/kg. In a preferred embodiment, Neutrokin-alpha is administered in a range of 0.01 to 3 mg/kg (specific preferred exemplary dosages in this embodiment include, but are not limited to, 0.01 mg/kg, 0.02 mg/kg, 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.06 mg/kg, 0.07 mg/kg, 0.08 mg/kg, 0.09 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1.0 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg, 1.6 mg/kg, 1.6 mg/kg, 1.7 mg/kg, 1.8 mg/kg, 1.9 mg/kg, 2.0 mg/kg, 2.1 mg/kg, 2.2 mg/kg, 2.3 mg/kg, 2.4 mg/kg, 2.5 mg/kg, 2.6 mg/kg, 2.7 mg/kg, 2.8 mg/kg, 2.9 mg/kg, and 3.0 mg/kg). In an additional preferred embodiment, Neutrokin-alpha is administered in a range of 0.02 to 2 mg/kg (specific preferred exemplary dosages in this embodiment include, but are not limited to, 0.02 mg/kg, 0.03 mg/kg, 0.04 mg/kg, 0.05 mg/kg, 0.06 mg/kg, 0.07 mg/kg, 0.08 mg/kg, 0.09 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1.0 mg/kg, 1.1 mg/kg, 1.2 mg/kg, 1.3 mg/kg, 1.4 mg/kg, 1.5 mg/kg, 1.6 mg/kg, 1.6 mg/kg, 1.7 mg/kg, 1.8 mg/kg, 1.9 mg/kg, and 2.0 mg/kg).

[0991] The data presented herein define Neutrokin-alpha, as a novel member of the TNF-ligand superfamily that induces both *in vivo* and *in vitro* B cell proliferation and differentiation. Neutrokin-alpha is distinguished from other B cell growth and differentiation factors such as IL2 (Metzger, D.W., *et al.*, *Res.Immunol.* 146:499-505 (1995)), IL4 (Armitage, R.J., *et al.*, *Adv.Exp.Med.Biol.* 292:121-30 (1991); Yokota, T., *et al.*, *Proc.Natl.Acad.Sci.U.S.A.* 83:5894-98 (1986)), IL5 (Takatsu, K., *et al.*, *Proc.Natl.Acad.Sci.U.S.A.* 84:4234-38 (1987); Bertolini, J.N., *et al.*, *Eur.J.Immunol.* 23:398-402 (1993)), IL6 (Poupart, P., *et al.*, *EMBO J.* 6:1219-24 (1987); Hirano, T., *et al.*, *Nature* 324:73-76 (1986)) IL7 (Goodwin, R.G., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 86:302-06 (1989); Namen, A.E., *et al.*, *Nature* 333:571-73 (1988)), IL13 (Punnonen, J., *et al.*, *Allergy* 49:576-86 (1994)), IL15 (Armitage, R.J., *et al.*, *J.Immunol.* 154:483-90 (1995)), CD40L (Armitage, R.J., *et al.*, *Nature* 357:80-82 (1992); Van Kooten, C. and

Banchereau, J. *Int.Arch.Allergy.Immunol.* 113:393-99 (1997)) or CD27L (CD70) (Oshima, H., *et al.*, *Int.Immunol.* 10:517-26 (1998); Lens, S.M., *et al.*, *Semin.Immunol.* 10:491-99 (1998)) by its monocyte-specific gene/protein expression pattern and its specific receptor distribution and biological activity on B lymphocytes. Taken together these data suggest that Neutrokin-alpha is likely involved in the exchange of signals between B cells and monocytes or their differentiated progeny. Although all B cells may utilize this mode of signaling, the restricted expression patterns and Ig secretion suggest a role for Neutrokin-alpha in the activation of CD5⁺ or "unconventional" B cell responses. These B cells provide a critical component to the innate immune system and provide protection from environmental pathogens through their secretion of polyreactive IgM and IgA antibodies (Pennell, C.A., *et al.*, *Eur.J.Immunol.* 19:1289-95 (1989); Hayakawa, K., *et al.*, *Proc.Natl.Acad.Sci.U.S.A.* 81:2494-98 (1984)). Alternatively, Neutrokin-alpha may function as a regulator of T cell independent responses in a manner analogous to that of CD40 and CD40L in T cell dependent antigen activation (van den Eertwegh, A.J., *et al.*, *J.Exp.Med.* 178:1555-65 (1993); Grabstein, K.H., *et al.*, *J.Immunol.* 150:3141-47 (1993)). As such, Neutrokin-alpha, its receptor or related antagonists have utility in the treatment of B cell disorders associated with autoimmunity, neoplasia and/or immunodeficient syndromes.

Methods

[0992] Mice. BALB/cAnNCR (6-8 weeks) were purchased from Charles River Laboratories, Inc. and maintained according to recommended standards (National Research Council, *Guide for the care and use of laboratory animals* (1999)) in microisolator cages with recycled paper bedding (Harlan Sprague Dawley, Inc., Indianapolis, IN) and provided with pelleted rodent diet (Harlan Sprague Dawley, Inc) and bottled drinking water on an ad libitum basis. The animal protocols used in this study were reviewed and approved by the HGS Institutional Animal Care and Use Committee.

[0993] Isolation of full length Neutrokin-alpha cDNA. The BLAST algorithm was used to search the Human Genome Sciences Inc. expressed sequence tag (EST) database for sequences with homology to the receptor-binding domain of the TNF family. A full length Neutrokin-alpha clone was identified, sequenced and submitted to GenBank (Accession number AF132600). The Neutrokin-alpha open reading frame was PCR

amplified utilizing a 5' primer (5'-CAG ACT GGA TCC GCC ACC ATG GAT GAC TCC ACA GAA AG-3') annealing at the predicted start codon and a 3' primer (5'-CAG ACT GGT ACC GTC CTG CGT GCA CTA CAT GGC-3') designed to anneal at the predicted downstream stop codon. The resulting amplicon was tailed with Bam HI and Asp 718 restriction sites and subcloned into a mammalian expression vector. Neutrokin-alpha was also expressed in p-CMV-1 (Sigma Chemicals).

[0994] Purification of recombinant human Neutrokin-alpha. The full length cDNA encoding Neutrokin-alpha was subcloned into the baculovirus expression vector pA2 and transfected into SF9 insect cells (Patel, V.P., *et al.*, *J.Exp.Med.* 185:1163-72 (1997)). Recombinant Neutrokin-alpha was purified from cell supernatants at 92 h post-infection using a combination of anion-exchange, size exclusion, and hydrophobic interaction columns. The purified protein was formulated in a buffer containing 0.15 M NaCl, 50 mM NaOAc at pH 6, sterile filtered and stored at 4°C until needed. Both SDS-PAGE and RP-HPLC analyses indicate that rNeutrokin-alpha is greater than 95% pure. Endotoxin levels were below the detection limit in the LAL assay (Associates of Cape Cod, Falmouth, MA). The final purified Neutrokin-alpha protein has an N-terminus sequence of Ala-Val-Gln-Gly-Pro. This corresponds identically to the sequence of soluble Neutrokin-alpha derived from CHO cell lines stably transfected with the full length Neutrokin-alpha gene.

[0995] Monoclonal antibody generation. BALB/cAnNCR mice were immunized with 50 micrograms of HisTag-Neutrokin-alpha suspended in complete Freund's adjuvant followed by 2 challenges in incomplete Freund's adjuvant. Hybridomas and monoclonal antibodies were prepared as described (Gefter, M.L., *et al.*, *Somatic Cell Genet.* 3:231-36 (1977); Akerstrom, B., *et al.*, *J.Immunol.* 135:2589-92 (1985)).

[0996] Cell lines. All human cell lines were purchased from ATCC (American Type Culture Collection, Manassas, VA).

[0997] FACS analysis. Neutrokin-alpha expression was assessed on human cell lines, freshly isolated normal peripheral blood nucleated cells, and in vitro cultured monocytes, a mouse anti-human Neutrokin-alpha mAb 2E5 (IgG1) followed by PE-conjugated F(ab')2 goat antibody to mouse IgG (CALTAG Laboratories, Burlingame, CA). Cells were analyzed using a FACScan (Becton Dickinson Immunocytometry Systems, San Jose, CA) with propidium iodide to exclude dead cells. Neutrokin-alpha

binding was assessed using rNeutrokin-alpha biotinylated with a N-hydroxysuccinimidobiotin reagent (Pierce, Rockford, IL) followed by PE-conjugated streptavidin (Dako Corp, Glostrup, Denmark).

[0998] Chromosomal mapping. To determine the chromosomal location of the Neutrokin-alpha gene, a panel of monochromosomal somatic cell hybrids (Quantum Biotechnology, Canada) retaining individual chromosomes was screened by PCR using Neutrokin-alpha specific primers (5' primer: 5'-TGG TGT CTT TCT ACC AGG TGG-3' and 3' primer: 5'-TTT CTT CTG GAC CCT GAA CGG-3'). The predicted 233 bp PCR product was only detected in human chromosome 13 hybrids. Using a panel of 83 radiation hybrids (Research Genetics, St. Louis, MO) and the Stanford Human Genome Center Database, (<http://www.shgc.stanford.edu.RH/rhserver>). Neutrokin-alpha was found linked to the SHGC-36171 marker on chromosome 13. Superposition of this map with the cytogenetic map of human chromosome 13 allowed the assignment of human Neutrokin-alpha to chromosomal band 13q34.

[0999] B lymphocyte proliferation assay. Human tonsillar B cells were purified by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population was routinely greater than 95% B cells as assessed by expression of CD19 and CD20. Various dilutions of human rNeutrokin-alpha or the control protein recombinant human IL2 were placed into individual wells of a 96-well plate to which was added 10^5 B cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5×10^{-5} M 2ME, 100U/ml penicillin, 100 microgram/ml streptomycin, and 10^{-5} dilution of Pansorbin (SAC) or anti-IgM) in a total volume of 150 microliters. Proliferation was quantitated by a 20h pulse (1 microCi/well) of 3 H-thymidine (6.7 Ci/mM) beginning 72h post factor addition.

[1000] Histological analyses. Spleens were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 micrometers, mounted on glass slides and stained with hematoxylin and eosin or by enzyme-labeled indirect method immunohistochemistry for CD45R(B220) (Hilbert, D.M., *et al.*, *Eur.J.Immunol.* 23:2412-18 (1993)).

Table V. Neutrokin-alpha cell surface expression

Cell line	Cellular Morphology	Neutrokin-alpha cell surface expression
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Monocytic lineage

U-937	Lymphoma, histiocytic/macrophage	+
BL-60	Leukemia, acutepromyelocytic	+
K-562	Leukemia, chronicmyelogenous	+
THP-1	Leukemia, acutemonocytic	+

T-lineage

Jurkat	Leukemia, T lymphocytic	-
SUP-T13	Leukemia, T lymphoblastic	-
MOLT-4	Leukemia, T lymphoblastic	-

B-lineage

Daudi	Burkitt's, lymphoblastic	-
Namalwa	Burkitt's, lymphocyte	-
Raji	Burkitt's, lymphocyte	-
Reh	Leukemia, lymphocytic	-
ARH-77	Leukemia, plasma cell	-
IM9	Myeloma	-
RPMI 8226	Myeloma	-

Example 7: Assays to detect stimulation or inhibition of B cell proliferation and differentiation

[1001] Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL5, IL6, IL-7, IL10, IL-13, IL14 and IL15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations. One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell

populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

[1002] *In Vitro* assay- Purified Neutrokin-alpha and/or Neutrokin-alphaSV protein, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of Neutrokin-alpha and/or Neutrokin-alphaSV protein on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220). Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added 10^5 B-cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5 X 10^{-5} M 2ME, 100U/ml penicillin, 10 ug/ml streptomycin, and 10^{-5} dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with 3 H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

[1003] Agonists (including Neutrokin-alpha and/or Neutrokin-alphaSV polypeptide fragments) demonstrate an increased B cell proliferation when compared to that observed when the same number of B cells is contacted with the same concentration of priming agent. Antagonists according to the invention exhibit a decreased B cell proliferation when compared to controls containing the same number of B cells, the same concentration of priming agent, and the same concentration of a soluble form of Neutrokin-alpha that elicits an increase in B cell proliferative activity (e.g., 71-285, 81-285, 112-285 or 134-285 of the Neutrokin-alpha polypeptide shown in SEQ ID NO:2) in the absence the antagonist.

[1004] *In Vivo* assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of Neutrokin-alpha and/or Neutrokin-alphaSV protein, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal and Neutrokin-alpha and/or Neutrokin-alphaSV protein-treated spleens identify the results of the activity of Neutrokin-alpha and/or Neutrokin-alphaSV protein on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

[1005] Flow cytometric analyses of the spleens from Neutrokin-alpha and/or Neutrokin-alphaSV protein-treated mice is used to indicate whether Neutrokin-alpha and/or Neutrokin-alphaSV protein specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

[1006] Likewise, a predicted consequence of increased mature B-cell representation *in vivo* is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and Neutrokin-alpha and/or Neutrokin-alphaSV protein-treated mice.

Example 8: Effect of Neutrokin-alpha and its agonists in treating graft-versus-host disease associated lymphoid atrophy and hypoplasia in mice

[1007] An analysis of the use of Neutrokin-alpha to treat, prevent, and/or diagnose graft-versus-host disease (GVHD)-associated lymphoid hypoplasia/atrophy is performed through the use of a C57BL/6 parent into (BALB/c X C57BL/6) F1 (CBF1) mouse model. This parent into F1 mouse model is a well-characterized and reproducible animal model of GVHD in bone marrow transplant patients, which is well known to one of ordinary skill in the art (see, Gleicher, *et al.*, *Immunol. Today* 5:324, 1984). Soluble Neutrokin-alpha is expected to induce the proliferation and differentiation of B lymphocyte, and correct

the lymphoid hypoplasia and atrophy observed in this animal model of GVHD (Piguet, *et al.*, *J. Exp. Med.* 166:1280 (1987); Hattori, *et al.*, *Blood* 90:542 (1997)).

[1008] Initiation of the GVHD condition is induced by the intravenous injection of approximately $1\text{-}5 \times 10^8$ spleen cells from C57BL/6 mice into (BALB/c X C57BL/6) F1 mice (both are available from Jackson Lab, Bar Harbor, Maine). Groups of 6 to 8 mice receive daily either 0.1 to 5.0 mg/kg of Neutrokin-alpha or buffer control intraperitoneally, intramuscularly or intradermally starting from the days when lymphoid hypoplasia and atrophy are mild (~day 5), moderate (~day 12) or severe (~day 20) following the parental cell injection. The effect of Neutrokin-alpha on lymphoid hypoplasia and atrophy of spleen is analyzed by FACS and histopathology at multiple time points (3-4) between day 10-30. Briefly, splenocytes are prepared from normal C57BL/6, GVHD or Neutrokin-alpha-treated mice, and stained with fluorescein phycoerythrin-conjugated anti- H-2K^b, biotin-conjugated anti- H-2K^d, and FITC-conjugated anti-CD4, anti-CD8, or anti-B220, followed by a CyChrome-conjugated avidin. All of these conjugated antibodies can be purchased from PharMingen (San Diego, CA). Cells are then analyzed on a FACScan (Becton Dickinson, San Jose, CA). Recipient and donor lymphocytes are identified as H-2K^b⁺ K^d⁺ and H-2K^b⁺ K^d⁻ cells, respectively. Cell numbers of CD4⁺T, CD8⁺ T and B220⁺ B cells of recipient or donor origin are calculated from the total numbers of splenocytes recovered and the percentages of each subpopulation are determined by the three color analysis. Histological evaluation of the relative degree of tissue damage in other GVHD-associated organs (liver, skin and intestine) may be conducted after sacrificing the animals.

[1009] Finally, Neutrokin-alpha and buffer-treated animals undergo a clinical evaluation every other day to assess cachexia, body weight and lethality.

[1010] Neutrokin-alpha agonists and antagonists may also be examined in this acute GVHD murine model.

Example 9. Isolation of antibody fragments directed against Neutrokin-alpha polypeptides from a library of scFvs.

[1011] Naturally occurring V-genes isolated from human PBLs are constructed into a large library of antibody fragments which contain reactivities against Neutrokin-alpha

and/or Neutrokinne-alphaSV to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein in its entirety by reference).

Rescue of the library.

[1012] A library of scFvs is constructed from the RNA of human PBLs as described in WO92/01047 (which is hereby incorporated by reference in its entirety). To rescue phage displaying antibody fragments, approximately 10^9 *E. coli* harboring the phagemid are used to inoculate 50 ml of 2x TY containing 1% glucose and 100 micrograms/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2×10^8 TU of delta gene 3 helper (M13 delta gene III, see WO92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2x TY containing 100 micrograms/ml ampicillin and 50 micrograms/ml kanamycin and grown overnight. Phage are prepared as described in WO92/01047.

[1013] M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37°C without shaking and then for a further hour at 37°C with shaking. Cells were spun down (IEC-Centra 8, 4000 revs/min for 10 min), resuspended in 300 ml 2x TY broth containing 100 micrograms ampicillin/ml and 25 micrograms kanamycin/ml (2x TY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 micrometer filter (Minisart NML; Sartorius) to give a final concentration of approximately 10^{13} transducing units/ml (ampicillin-resistant clones).

Panning the Library.

[1014] Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 micrograms/ml or 10 micrograms/ml of a polypeptide of the present invention. Tubes are

blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 10¹³ TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log *E. coli* TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The *E. coli* are then plated on TYE plates containing 1% glucose and 100 micrograms/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders.

[1015] Eluted phage from the third and fourth rounds of selection are used to infect *E. coli* HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtiter plates coated with either 10 picograms/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see e.g., WO92/01047) and then by sequencing.

Example 10. Neutralization of Neutrokinne-alpha/Neutrokinne-alpha Receptor Interaction with an anti-Neutrokinne-alpha Monoclonal Antibody.

[1016] Monoclonal antibodies were generated against Neutrokinne-alpha protein according to the following method. Briefly, mice were given a subcutaneous injection (front part of the dorsum) of 50 micrograms of His-tagged Neutrokinne-alpha protein produced by the method of Example 2 in 100 microliters of PBS emulsified in 100 microliters of complete Freunds adjuvant. Three additional subcutaneous injections of 25 micrograms of Neutrokinne-alpha in incomplete Freunds adjuvant were given at 2-week intervals. The animals were rested for a mounth before they received the final

intraperitoneal boost of 25 micrograms of Neutrokinne-alpha in PBS. Four days later mice were sacrificed and splenocytes taken for fusion.

[1017] The process of "Fusion" was accomplished by fusing splenocytes from one spleen with 2x10E7 P3X63Ag8.653 plasmacytoma cells using PEG 1500 (Boehringer Mannheim), according to the manufacturer's modifications of an earlier described method. (See, Gefter, M.L., *et al. Somatic Cell Genet* 3:231-36 (1977); Boehringer Mannheim, PEG 1500 (Cat.No. 783641), product description.)

[1018] After fusion, the cells were resuspended in 400 ml of HAT medium supplemented with 20% FBS and 4% Hybridoma Supplement (Boehringer Mannheim) and distributed to 96 well plates at a density of 200 microliters per well. At day 7 post-fusion, 100 microliters of medium was aspirated and replaced with 100 microliters of fresh medium. At day 14 post-fusion, the hybridomas were screened for antibody production.

[1019] Hybridoma supernatants were screened by ELISA for binding to Neutrokinne-alpha protein immobilized on plates. Plates were coated with Neutrokinne-alpha by overnight incubation of 100 microliters per well of Neutrokinne-alpha in PBS at a concentration of 2 micrograms per ml. Hybridoma supernatants were diluted 1:10 with PBS were placed in individual wells of Neutrokinne-alpha-coated plates and incubated overnight at 4°C. On the following day, the plates were washed 3 times with PBS containing 0.1% Tween-20 and developed using the anti-mouse IgG ABC system (Vector Laboratories). The color development reaction was stopped with the addition of 25 ml/well of 2M H₂SO₄. The plates were then read at 450 nm.

[1020] Hybridoma supernatants were checked for Ig isotype using Isostrips. Cloning was done by the method of limiting dilutions on HT medium. About 3x10E6 cells in 0.9 ml of HBSS were injected in pristane-primed mice. After 7-9 days, ascitic fluid was collected using a 19 g needle. All antibodies were purified by protein G affinity chromatography using the Acta FPLC system (Pharmacia).

[1021] After primary and two consecutive subcutaneous injections, all three mice developed a strong immune response; the serum titer was 10E-7 as assessed by ELISA on Neutrokinne-alpha-coated plates.

[1022] In one experiment, using the splenocytes from the positive mouse more than 1000 primary hybridomas were generated. 917 of them were screened for producing anti-Neutrokinne-alpha antibody. Screening was performed using 1:1 diluted supernatants in

order to detect all positive clones. Of 917 hybridomas screened, 76 were found to be positive and 17 of those were found to be IgG producers. After affinity testing and cloning, 9 of them were chosen for further expansion and purification.

[1023] All purified monoclonal antibodies were able to bind different forms of Neutrokin-alpha (including His-tagged and protein produced from a baculoviral system (see Example 2)) in both Western blot analysis and ELISA. Six of nine clones were also able to bind Neutrokin-alpha on the surface of THP-1 cells. However, none of the antibodies tested were able to capture Neutrokin-alpha from solution.

[1024] High affinity anti-Neutrokin-alpha monoclonal antibodies were generated that recognize Neutrokin-alpha expressed on the cell surface but not in solution can be used for neutralization studies *in vivo* and in monocyte and B cell assays *in vitro*. These antibodies are also useful for sensitive detection of Neutrokin-alpha on Western blots.

[1025] In an independent experiment, using the splenocytes from the positive mouse, more than 1000 primary hybridomas were generated. 729 of the primary hybridomas were then screened for the production of an anti-Neutrokin-alpha antibody. Screening was performed under stringent conditions using 1:10 diluted supernatants in order to pick up only clones of higher affinity. Of 729 hybridomas screened, 23 were positive, including 16 IgM and 7 IgG producers (among the latter, 4 gave a strong IgM background). In this experiment, the isotype distribution of IgG antibodies was biased towards the IgG2 subclasses. Three of seven IgG hybridomas produced antibodies of IgG2a subclass and two produced an antibody of IgG2b subclass, while the remaining two were IgG1 producers.

[1026] Supernatants from all positive hybridomas generated in the second experiment were tested for the ability to inhibit Neutrokin-alpha-mediated proliferation of B cells. In the first screening experiment, two hybridomas producing IgG-neutralizing antibodies were detected (these are antibodies 16C9 and 12C5). In additional experiments, the IgG-neutralizing activity of the hybridomas (i.e., 16C9 and 12C5) were confirmed and two additional strongly neutralizing supernatants from hybridomas 15C10 and 4A6 were identified.

[1027] Three clones were subsequently expanded *in vivo* (a single clone, i.e., 15C10, was also expanded in a hollow fiber system), and the antibody purified by affinity chromatography. All three of the clones were able to bind Neutrokin-alpha on the

surface of THP-1 cells and were also able to bind (i.e., "capture") Neutrokin-alpha from solution.

[1028] Specifically, experiments were performed using the anti-Neutrokin-alpha monoclonal antibodies described in the second experiment above to determine whether the antibodies neutralize Neutrokin-alpha/Neutrokin-alpha Receptor binding. Briefly, Neutrokin-alpha protein was biotinylated using the EZ-link T NHS-biotin reagent (Pierce, Rockford, IL). Biotinylated Neutrokin-alpha was then used to identify cell surface proteins that bind Neutrokin-alpha. Preliminary experiments demonstrated that Neutrokin-alpha binds to a receptor on B lymphoid cells.

[1029] The inclusion of anti-Neutrokin-alpha antibodies generated in the second experiment described above neutralized binding of Neutrokin-alpha to a Neutrokin-alpha receptor. In a specific embodiment, anti-Neutrokin-alpha antibody 15C10 neutralizes binding of Neutrokin-alpha to a Neutrokin-alpha Receptor.

[1030] Thus, the anti-Neutrokin-alpha monoclonal antibodies generated in the second experiment described above (in particular, antibody 15C10) recognize and bind to both membrane-bound and soluble Neutrokin-alpha protein and neutralize Neutrokin-alpha/Neutrokin-alpha Receptor binding *in vitro*.

Example 11. Neutrokin-alpha induced signalling in B cells

[1031] Total RNA was prepared from tonsillar B cells unstimulated or stimulated with SAC or SAC plus soluble Neutrokin-alpha (amino acids 134-285 of SEQ ID NO:2, 100ng/mL) for 12 hours. Messenger RNA levels of ERK-1 and PLK was determined by real time quantitative PCR using ABI 7700 Taqman sequence detector. Amplification primers and probes were designed to span the region from nucleotides 252-332 of the human PLK sequence and nucleotides 373 to 446 of the human ERK-1 mRNA (Genbank accession numbers X75932 and X60188, respectively). For quantitation of RNA, the comparative delta CT method was used (Perkin-Elmer user Bulletin #2 and #4, 1997) using an 18S ribosomal RNA probe as endogenous reference. Expression levels were characterized relative to observed levels in unstimulated B-cells.

Example 12. Rapid and Specific Targeting of Radiolabeled Neutrokin-alpha to Lymphoid Tissues

[1032] Here, biodistribution studies of radiolabeled Neutrokin-alpha are reported that demonstrate high *in vivo* targeting specificity of Neutrokin-alpha for lymphoid tissues. Neutrokin-alpha was radiolabeled with ^{125}I and injected intravenously into BALB/c mice. Three doses and 4 timepoints over a 24-hr period were studied. Biodistribution was measured by direct counting of the radioactivity in dissected whole organs or tissues and by whole body quantitative autoradiography (QAR).

[1033] Spleen and lymph nodes showed the highest concentration of radioactivity among the dissected organs and tissues. Three hr after injection of 0.01 mg/kg Neutrokin-alpha, 63% and 23% injected dose (ID)/g were measured in spleen and lymph node, respectively, compared to ~5% for both kidney and liver. As the dose was increased, the %ID/g in spleen and lymph node decreased but was unchanged in liver and kidney, suggesting that targeting to spleen and lymph nodes is mediated by saturable binding. With increasing time, the ratio of the concentration in spleen and lymph node to the concentration in either kidney or liver increased. QAR confirmed the high uptake of radiolabeled Neutrokin-alpha in spleen and lymph nodes at 3 hr, and revealed high uptake in bone marrow, gut-associated lymphoid tissue (GALT) and intestinal contents as well. At 24 hr, spleen, lymph nodes and GALT were still strongly positive for radiolabeled Neutrokin-alpha by QAR whereas liver and kidney no longer had observable levels. A cytotoxic radionuclide coupled to Neutrokin-alpha could irradiate neoplastic B-cells trafficking through or residing in lymphoid tissues. Thus, the rapid and highly specific targeting of radiolabeled Neutrokin-alpha to lymphoid tissues provides a rationale for its application in the treatment of B-cell malignancies.

Example 13: Pharmacological Effects of ^{131}I -labeled Neutrokin-alpha in BCL1 Tumor-Bearing Mice and J558 Tumor Bearing Mice

^{131}I -Neutrokin-alpha administration to BCL1 Tumor-Bearing Mice

[1034] The BCL1 tumor cell line was derived from a spontaneous murine B cell tumor. Intraperitoneal inoculation of the BCL1 cell line in BALB/c mice results in splenomegaly, and subsequent death. The BCL1 tumor cell phenotype is IgM positive, complement receptor negative, Fc receptor positive and has marginal IgD expression (Knapp et al., J. Immunol. 123:992-999 (1979) and Vitetta et al. Blood 89:4425-36.

(1997)). Based on FACS analysis using biotinylated Neutrokinne-alpha, BCL1 cells freshly isolated from the spleens of BALB/c mice express Neutrokinne-alpha receptors on their cell surface. The BCL1 tumor model is a relevant mouse model for human B cell lymphoma, providing a means to test the ability of ^{131}I -labeled Neutrokinne-alpha to kill leukemic B cells and consequently prolong survival of tumor-bearing mice. Three lots of ^{131}I -labeled Neutrokinne-alpha (Lots TX1, TX2 and TX3) were prepared by MDS Nordion (Ontario, Canada) and used in 3 different experiments to evaluate the effects of ^{131}I -labeled Neutrokinne-alpha in this murine model.

[1035] Female BALB/c mice were injected intraperitoneally (ip) on Day 0 with 1 x 10^5 viable BCL1 cells that had been propagated in vivo. Treatment groups for the 3 experiments are described in Table VI. Ten days after injection of tumor cells, the animals were administered ^{131}I -labeled Neutrokinne-alpha iv in 110 μL . The doses administered were 11.9 or 15.3 mCi/kg (TX1), 17.5 mCi/kg (TX2), or 37.7 mCi/kg (TX3) for the 3 experiments. To identify potentially toxic effects of the administered ^{131}I -labeled Neutrokinne-alpha, age-matched control BALB/c mice without BCL1 tumors were injected with identical doses of the ^{131}I -labeled protein. An additional group of BALB/c mice, bearing BCL1 tumors and receiving an iv injection of the vehicle, served as the normal tumor control group. Survival was then monitored for 48, 44, or 40 days for the TX1, TX2, and TX3 experiments, respectively.

Table VI Treatment groups for TX1, TX2 and TX3 experiments

<u>Exp.</u>	<u>Group</u>	^{131}I -Neutrokinne-alpha Dose (mCi/kg)	<u>n</u>	BCL1 Tumor Inoculated ip (No. of cells)
1 (TX1)	1 Vehicle	0	15	1×10^5
	2 ^{131}I -Neutrokinne-alpha	11.9	10	1×10^5
	3 ^{131}I -Neutrokinne-alpha	15.3	10	1×10^5
	4 ^{131}I -Neutrokinne-alpha	11.9	10	0
	5 ^{131}I -Neutrokinne-alpha	15.3	10	0
2 (TX2)	1 Vehicle	0	12	1×10^5
	2 ^{131}I -Neutrokinne-alpha	17.5	12	1×10^5
	3 ^{131}I -Neutrokinne-alpha	17.5	8	0
3 (TX3)	1 Vehicle	0	14	1×10^5
	2 ^{131}I -Neutrokinne-alpha	37.7	14	1×10^5
	3 ^{131}I -Neutrokinne-alpha	37.7	8	0

[1036] The endpoint monitored in the 3 experiments was survival (days) following ip inoculation of BCL1 tumor cells. All animals were examined daily. The day post-inoculation that mice were either found dead or in moribund condition (the latter being immediately euthanized for humane reasons) was recorded.

[1037] A single iv administration of either 11.9 or 15.3 mCi/kg (TX1), 17.5 mCi/kg (TX2), or 37.7 mCi/kg (TX3) of ¹³¹I-labeled Neutrokinne-alpha injected 10 days after intraperitoneal inoculation of BCL1 cells in BALB/c mice significantly improved survival compared with mice inoculated with tumor and treated with the ¹³¹I-labeled Neutrokinne-alpha vehicle (Figures 12-14; in Figures 12-14, ¹³¹I-labeled Neutrokinne-alpha is indicated as LR131). The median survival time for the vehicle-treated, tumor-bearing mice was 18, 21, and 19 days post-tumor cell injection for the TX1, TX2, and TX3 experiments, respectively. In the TX1 experiment, ¹³¹I-labeled Neutrokinne-alpha administration at dose levels of 11.9 and 15.3 mCi/kg doubled the median survival time of tumor-bearing mice to 35.5 (11.9 mCi/kg) and 34 (15.3 mCi/kg) days post-treatment, respectively. In the TX2 and TX3 experiments, ¹³¹I-labeled Neutrokinne-alpha administration at a dose of 17.5 or 37.7 mCi/kg increased the median survival time of tumor-bearing mice to 30 and 22 days post-treatment, respectively. Tumor-bearing mice treated with all doses of ¹³¹I-labeled Neutrokinne-alpha in the 3 experiments had a significantly lower risk of dying than tumor-bearing mice treated with vehicle (Table VII).

Table VII Incidence of mortality for TX1 – TX3 experiments

<u>Experiment</u>	<u>Treatment Group</u>	<u>Median Survival Time (Days)</u>
TX1	1, BCL1 + ¹³¹ I-labeled Neutrokinne-alpha (11.9 mCi/kg)	35.5
	2, BCL1 + ¹³¹ I-labeled Neutrokinne-alpha (15.3 mCi/kg)	34
	3, BCL1 Tumor Only	18
	4, No Tumor + ¹³¹ I-labeled Neutrokinne-alpha (11.9 mCi/kg)	> 48
	5, No Tumor + ¹³¹ I-labeled Neutrokinne-alpha (15.3 mCi/kg)	> 48
TX2	1, BCL1 + ¹³¹ I-labeled Neutrokinne-alpha (17.5 mCi/kg)	30
	2, BCL1 Tumor Only + vehicle	21
	3, No Tumor + ¹³¹ I-labeled Neutrokinne-alpha (17.5 mCi/kg)	> 44
TX3	1, BCL1 + vehicle	19
	2, BCL1 + ¹³¹ I-labeled Neutrokinne-alpha (37.7 mCi/kg)	22
	3, No tumor + ¹³¹ I-labeled Neutrokinne-alpha (37.7 mCi/kg)	> 40

[1038] In the TX1 – TX3 series of experiments, the effect that increasing the dose of ¹³¹I-labeled Neutrokin-alpha had on the survival of the BCL1 tumor-bearing animals was investigated. A maximal survival benefit was achieved with the low doses of ¹³¹I-labeled Neutrokin-alpha (11.9 and 15.3 mCi/kg). The much reduced effectiveness of ¹³¹I-labeled Neutrokin-alpha in TX3 may be due to toxicity associated with the high dose of the material used.

[1039] In conclusion, a single iv administration of ¹³¹I-labeled Neutrokin-alpha administered to mice bearing BCL1 leukemia cell splenic tumors significantly improved survival compared with tumor-bearing mice treated with vehicle.

¹³¹I-Neutrokin-alpha administration to J558 Tumor-Bearing Mice

[1040] In a similar experiment as that described above, BALB/c mice were injected subcutaneously with J558 plasmacytoma cells (ATCC # TIB-6) and treated with a single intravenous treatment of 25mCi/kg of ¹³¹I-labeled Neutrokin-alpha. 24 BALB/c mice (NCI, 4 weeks old, average weight 18 g) were divided into 2 groups (12 mice per group) and injected sc with 2.5×10^5 J558 cells in 100 mL of PBS. At Day 9 after injection, mice in Group 1 were injected intravenously with 100 mL of formulation buffer, and mice in Group 2 were injected iv with a dose of 25 mCi/kg of ¹³¹I-Neutrokin-alpha in 100 mL of formulation buffer. The average body weight at the time of ¹³¹I-Neutrokin-alpha injection was 19.5 g.

[1041] Two parameters were evaluated during this study the tumor size and the time to tumor response. To evaluate tumor size the short and long axes of the tumor were measured using an electronic digital caliper. Tumor size was calculated by multiplication of the lengths of the short and long axes and expressed in mm². The time to tumor response was characterized by the day after cell inoculation when a visible tumor (> 2mm) was detected on a mouse. In addition, mice were monitored for survival and signs of radiation induced toxicity (general appearance, activity, breathing frequency, stool consistence).

[1042] One mouse in the ¹³¹I-Neutrokin-alpha-treated group died on Day 25 (16 days after ¹³¹I-Neutrokin-alpha treatment) with no obvious signs of radiation related toxicity.

A second mouse died in the same group on Day 30, when all animals in the control group were terminated because of large tumor size.

[1043] The first tumors of measurable size were detected at Day 14 in the buffer control group, where 4 out of 12 animals developed tumors. In the ^{131}I -Neutrokin-alpha treated animals, tumor formation was delayed by 6 days. Only one mouse out of 12 developed a tumor at Day 20. At Day 22, there was only one tumor-bearing mouse in the ^{131}I -Neutrokin-alpha treated group out of 12 animals, whereas in the buffer control group, 11 out of 12 mice developed tumors of different sizes. At Day 27, the mean tumor size in the buffer control group was 489 mm^2 (all tumor positive mice in this group were terminated at this time point). In the ^{131}I -Neutrokin-alpha treated group, the mean tumor size was 32.7 mm^2 , 15 times smaller than in the buffer control group. Taken together, these data suggest a strong inhibition of J558 tumor development in mice treated with ^{131}I -Neutrokin-alpha at a dose of 25 mCi/kg and tumor load of 2.5×10^5 cells/mouse.

[1044] In conclusion, a single intravenous administration of ^{131}I -Neutrokin-alpha into BALB/c mice at a dose of 25 mCi/kg significantly inhibits subcutaneous growth of J558 plasma cell tumors. At the initial tumor load of 2.5×10^5 cells/mouse, a 6 day delay in tumor formation and a 15-fold reduction in tumor size was observed in ^{131}I -Neutrokin-alpha treated animals.

[1045] The anti-neoplastic effects of ^{131}I -Neutrokin-alpha were accompanied by the expected B lymphocyte hypoplasia and a transient (<20 days) depletion of cKit $^+$ bone marrow precursors and peripheral platelets. Peripheral neutrophil, red blood cell, and monocyte counts were unaffected by ^{131}I -Neutrokin-alpha treatment. Taken together, the results demonstrate that ^{131}I -Neutrokin-alpha inhibits in vivo tumor growth in two models of B cell neoplasia. Moreover, ^{131}I -Neutrokin-alpha efficacy was not accompanied by significant bone marrow toxicities or peripheral myelosuppression.

EXAMPLE 14: Improved Method for Producing Neutrokin-alpha Using a Stringent Promoter and Low Expression Level

[1046] Neutrokin-alpha has been produced in *Escherichia coli* K-12 from the periplasmic fraction of the cell lysate. Using this system, soluble, properly folded, active Neutrokin-alpha is not obtainable from simple shake flask experiments. Yields of

soluble Neutrokin-alpha from complex media fermentations in small and large-scale bioreactors are on the order of 1-5 mg/L. Greater yields (25-38 mg/L) of soluble, properly folded, active Neutrokin-alpha can be accomplished in bioreactors at low to medium cell density under defined medium conditions. Moreover, this low quantity of protein is difficult to purify via conventional methods.

[1047] This example describes a method for the production of high yields of soluble, properly folded, active Neutrokin-alpha in the periplasm of *Escherichia coli*, which permits the use of conventional methods for Neutrokin-alpha purification, such as those described below or in Example 2 (paragraphs [0938] -[0948] , with modifications for *E. coli*, as would be apparent to one of ordinary skill in the art). Additionally, Neutrokin-alpha protein may be purified using affinity columns comprising Neutrokin-alpha binding peptides such as those described in WO 02/02641, which is herein incorporated by reference in its entirety. Purified Neutrokin-alpha may be quantified using RP-HPLC.

[1048] This method relies on the expression of Neutrokin-alpha protein from the bacterial *phoA* promoter. The *phoA* promoter is a very tightly regulated system that exhibits a very low level of transcription in the presence of excess phosphate. As the phosphate level in the medium decreases below a threshold of ~4 micromolar (Wanner, B.L., *J Cell Biochem* 51:47 (1993)), transcription is induced about 1000-fold. The *phoA* promoter yields a gradual build-up of recombinant protein, instead of a sharp increase of induction that occurs with other systems. This gradual or steady increase in recombinant protein minimizes the chance of overwhelming the components of the bacterial expression system and may also minimize the formation of inclusion bodies. Furthermore, this gradual build up permits the expression of proteins that might have been toxic to the cell if they were induced to high levels over a short period of time.

Expression Vector pML124

[1049] The expression vector, pML124, was created using pBR322 as the starting backbone. First, the endogenous NdeI site of pBR322 was eliminated by digesting it with NdeI, filling in the overhanging ends with the Klenow enzyme, then re-ligating the two blunt-ends back together (this created pML123). Next, pML123 was digested with EcoRI and BamHI restriction enzymes and the linear plasmid (loss of ~375 bp of DNA) was agarose gel purified (Qiagen).

[1050] The *phoA* promoter region was PCR-amplified from the *E. coli* K-12 chromosome (W3110; ATCC Catalogue No. 27325) with EcoRI (5') and BamHI (3') engineered sites. NdeI and KpnI sites were also engineered downstream of the *phoA* promoter to facilitate cloning of recombinant genes. Finally, the Shine-Dalgarno (SD) box was optimized for protein expression. The wild-type SD box and its adjacent sequence is as follows (the putative SD boxes are underlined and in bold):

5'-TTTGTACATGGAGAAAATAAA (SEQ ID NO:56):-[ATG, start of coding sequence]-3'

Optimized SD box and adjacent sequence is as follows:

5'-CACGTAAAGGAAGTTCTCAT (SEQ ID NO:57)-[ATG, start of coding sequence]-3'

[1051] The digested (EcoRI and BamHI) and purified *phoA* promoter PCR product was ligated into the agarose gel purified pML123 (described above). The ligation mixture was transformed into highly competent *E. coli* cells using standard techniques. Positive clones were identified via restriction analysis and DNA sequencing.

[1052] pML124 contains a gene for ampicillin resistance, a ColE1 replicon (pBR322-based), Rop, *phoA* promoter, the optimized Shine-Dalgarno (SD) box (above) and a multiple cloning site. Figure 15 is a plasmid map of pML124 and SEQ ID NO:52 is the nucleotide sequence of pML124. Additionally, plasmid pML124 was deposited at the American Type Culture Collection (ATCC) on October 8, 2001 and given ATCC Deposit No. PTA-3778. ATCC Deposit Nos. PTA-3778 was made pursuant to the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The ATCC (American Type Culture Collection) is located at 10801 University Boulevard, Manassas, Virginia 20110-2209.

Neutrokine-alpha Expression Vector pML124-MBPssBLyS

[1053] A fusion construct of the maltose binding protein signal sequence (MBPss) and Neutrokine-alpha was placed behind the *phoA* promoter in pML124 as follows. A 549 bp NdeI/KpnI MBPss-Neutrokine-alpha containing DNA insert was ligated into NdeI/KpnI

digested and gel purified pML124 to form pML124-MBPss-BLyS. (Figures 16, SEQ ID NO:53 ATCC Deposit No. PTA-3867, deposited November 16, 2001). ATCC Deposit No. PTA-3867 was made pursuant to the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The ATCC (American Type Culture Collection) is located at 10801 University Boulevard, Manassas, Virginia 20110-2209.

[1054] The pML124 plasmid (Figures 15, SEQ ID NO:52) is described in above and in U.S. Provisional Applications 60/329,508 filed October 17, 2001, 60/329,747 filed October 18, 2001 and 60/331,478 filed November 16, 2001 which are herein incorporated by reference in their entireties. The *phoA* promoter region is located at nucleotides 111-410 SEQ ID NOs:52 and 53. The MBP signal sequence is encoded by nucleotides 423-500 of SEQ ID NO:53 and nucleotides 501-959 of SEQ ID NO:53 encode amino acids 134-285 of Neutrokine-alpha (SEQ ID NO:2). The amino acid sequence of the MBP signal sequence is shown in SEQ ID NO:54 and the amino acid sequence of the full length MBP signal sequence-neutrokine-alpha protein encoded by the pML124-MBPss-BLyS vector is shown in SEQ ID NO:55

Neutrokine-alpha Expression in E. coli

[1055] Plasmid pML124-MBPss-BLyS was transformed into *E. coli* cells, e.g. K-12 based strains, by standard methods. Ampicillin resistant transformants were screened for the proper DNA insert by restriction enzyme analysis and DNA sequence. For example, digestion of pML124-MBPss-BLyS™ with NdeI and KpnI results in two nucleotide fragments: 549 and 4,431 base pairs in length. Positive clones were subsequently grown in City Broth-Low Phosphate media (see recipe below). Neutrokine-alpha expression levels were examined via SDS-PAGE and subsequent Coomassie staining. Using simple shake flask experiments, more than 260 mg/L of Neutrokine-alpha was obtained.

[1056] Next, positive clones were grown to high cell density in complex media in small scale bioreactors, similar to the method described by Joly *et al.*, *PNAS* 95:2773-2777 (1998), which is hereby incorporated by reference in its entirety. Specifically, the initial fermentation medium for the 5L bioreactor was composed of 55.7 mM ammonium sulfate, 13.9 mM sodium monobasic phosphate, 21.9 mM potassium dibasic phosphate, 5 mM sodium citrate, 29.6 mM potassium chloride, 14.7 mM magnesium sulfate, 1.11%

NZ-amine AS, 1.11% yeast extract, 5 g/L glucose, 0.002% ferric chloride, 25 µg/ml kanamycin. A trace element solution (2.5 ml/3.4 L) was added containing 100 mM ferric chloride plus 30 mM of the following components: zinc sulfate, cobalt chloride, sodium molybdate, copper sulfate, boric acid and manganese sulfate. The fermenter was operated at 30°C, 650 rpm agitation, 10 standard liter/minute aeration. When the initial glucose was depleted, a concentrated glucose solution (50%) was added until the dissolved oxygen (DO) concentration reached 20% of air saturation as measured by an on-line oxygen electrode. When the optical density (600 nm) reached 40 OD₆₀₀, a solution of 20% NZ amine AS, 20% yeast extract was fed at 0.2 ml/min for the rest of the fermentation. Neutrokine-alpha production was on the order of 260-570mg/L.

Low Phosphate Containing Media:

City Broth-Low Phosphate:

30mM (NH₄)₂SO₄; 2.25 mM NaCitrate-2H₂O; 12mM MgSO₄; 15 mM KCl; 5% Yeast extract; 2% Casamino acids; 110 mM MOPS; 33 mM Glucose; pH 7.3

Vegan City Broth-Low Phosphate:

30mM (NH₄)₂SO₄; 2.25 mM NaCitrate-2H₂O; 12mM MgSO₄; 15 mM KCl; 5% Phytone; 2% Casamino acids; 110 mM MOPS; 33 mM Glucose; pH 7.3

[1057] The only difference between the two media is that Phytone is substituted for Yeast extract in the Vegan recipe.

Purification of Neutrokine-alpha

[1058] 10 grams of *E. coli* cell paste are suspended in 50 milliliters of 5mM sodium citrate, pH 6.0 and placed at 4°C for 1 hour with gentle shaking. Cells are then disrupted by passing them through an M-Y110 Microfluidizer® Processor (Microfluidics, Inc., Newton, MA) set at 7500 psi four times. The suspension is then centrifuged at 22,000 x g for twenty minutes at 4°C using a Sorvall SLA-1500 rotor. The supernatant is then collected and filtered through a 0.45 micron bottle top filter (Nalgene).

[1059] Filtered supernatant is then loaded at 9 centimeters/hour on a Fast Flow Sepharose DEAE column (Amersham Biosciences, Piscataway, NJ) previously equilibrated with 5mM sodium citrate, pH6.0 (equilibration buffer). After loading, the column is washed with 5 to 10 column volumes of equilibration buffer. The Neutrokin-alpha protein is eluted with a 200mM NaCl step in equilibration buffer. Buffers used with the Fast Flow Sepharose DEAE chromatography column are pre-filtered using a 0.22 micron CA bottle top filter (Nalgene) and pre-chilled to 4°C. The Fast Flow Sepharose DEAE column is used at 4°C. Prior to use, columns are cleaned with 0.5 M NaOH.

[1060] Relevant fractions, as determined by the ratio of contaminating proteins to Neutrokin-alpha protein seen in Coomassie stained SDS-PAGE gels, are pooled and diluted 1:1 with 10mM sodium citrate, pH 6.0, 2M (NH₄)SO₄. Pooled fractions are loaded at 17 centimeters/hour onto a Polypropylene Glycol Hydrophobic Interaction chromatography column (Tosoh Biosep, Montgomeryville, PA) previously equilibrated with 10mM sodium citrate, pH 6.0, 1M (NH₄)SO₄ (loading buffer). After loading, the column is washed with 5-10 volumes of loading buffer. The Neutrokin-alpha protein is eluted with a 5 column volume gradient from loading buffer to elution buffer (10mM sodium citrate, pH 6.0). Neutrokin-alpha elutes in the second peak toward the end of the gradient absorbance at 280nm. Buffers used with the Polypropylene Glycol Hydrophobic Interaction chromatography column are pre-filtered using a 0.22 micron CA bottle top filter (Nalgene) and used at room temperature. The Polypropylene Glycol Hydrophobic Interaction chromatography column is also used at room temperature. Prior to use, columns are cleaned with 0.5 M NaOH.

[1061] Relevant fractions, determined by the ratio of contaminating proteins to Neutrokin-alpha protein as monitored by Coomassie stained SDS-PAGE gels, are pooled and are dialyzed overnight (12 hours) into 50mM Tris, pH 7.4, 50mM NaCl at 4°C. The dialyzed pool is then loaded onto a POROS PI-50 anion exchange chromatography column (Applied Biosystems, Foster City, CA), previously equilibrated with 50mM Tris, pH 7.4, 50mM NaCl, at 17 centimeters/hour. After loading, column is washed with 5-10 volumes of loading buffer. Neutrokin-alpha is eluted using a pH step from 50mM Tris, pH 7.4, 50mM NaCl buffer to 50mM sodium citrate, pH 6.0. Relevant fractions, as determined by the ratio of contaminating proteins to Neutrokin-alpha protein seen in Coomassie stained SDS-PAGE gels, are pooled and stored at 4°C. Buffers used with the

POROS PI-50 anion exchange chromatography column are pre-filtered using a 0.22 micron CA bottle top filter (Nalgene) and pre-chilled to 4°C. The POROS PI-50 anion exchange chromatography column is used at 4°C. Prior to use, columns are cleaned with 0.5 M NaOH.

[1062] This purification protocol yields 0.5-1 milligram per gram of starting cell paste based on BCA protein assay (Pierce Biotechnology, Rockford, IL) and absorbance at 280 nanometers. The protein is 96% pure as determined by reverse phase-high performance liquid chromatography (RP-HPLC). Native-PAGE and size exclusion chromatography-HPLC (SEC-HPLC) analysis indicates the protein is predominantly in trimeric form.

[1063] The production of MBPss-Neurokine-alpha under control of the *phoA* promoter allowed more stringent, slower expression, and resulted in increased yields. In summary, the production of Neurokine-alpha from the *phoA* system is scaleable and achieves 10 to 20-fold more soluble, properly folded, active material than the current system.

Example 15: Competitive Binding Studies between antibody 15C10 and 3D4.

[1064] To determine if antibodies 15C10 and 3D4 bind similar or distinct epitopes, competitive binding studies were performed.

[1065] Soluble Neurokine-alpha (amino acids 134-284 of SEQ ID NO:2) was preincubated with 15C10 or 3D4 antibodies. Hereinafter in this example, the antibody with which Neurokine-alpha was preincubated will be referred to as the "competing antibody". After preincubation, soluble Neurokine-alpha-competing antibody complexes were captured on an ELISA plate coated with either 3D4 or 15C10. Hereinafter in this example, the antibody coated on the ELISA plate will be referred to as the "capture antibody". After binding, and wash steps, soluble Neurokine-alpha-competing antibody complexes captured on the 3D4 or 15C10-coated ELISA plates was detected using a biotinylated polyclonal anti-Neurokine-alpha antibody followed by a streptavidin-coupled detection agent such as horse radish peroxidase or alkaline phosphatase.

[1066] If there is no competition between the competing antibody and the capture antibody on the ELISA plate (i.e., if the two antibodies bind non-overlapping epitopes), soluble Neurokine-alpha will be not prevented from binding to the capture antibody on

the ELISA plate and the ELISA will give a positive signal. On the other hand, if there is competition between the competing antibody and the capture antibody on the ELISA plate (i.e., if the two antibodies bind overlapping or identical epitopes), a decreased (or no) amount of soluble Neutrokin-alpha will be bound to the ELISA plate and the ELISA will give a decreased signal, compared to the signal given in the absence of competition between the two antibodies.

[1067] When an assay similar to that described above was performed using monoclonal antibodies 15C10 and 3D4, it was found that the two antibodies competed with each other, irrespective of which antibody was the competing antibody and which antibody was the capture antibody. These results indicate that 15C10 and 3D4 at least have overlapping epitopes. Isotype matched controls of irrelevant specificity (non-Neutrokin-alpha binding) were not able to compete for binding

[1068] It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

[1069] The entire disclosure of all publications (including patents, patent applications, journal articles, laboratory manuals, books, or other documents) cited herein are hereby incorporated by reference.

[1070] Further, the Sequence Listing submitted herewith in both computer and paper forms are hereby incorporated by reference in their entireties. Additionally, the entire disclosure (including the specification, sequence listing, and drawings) of each of the following U.S. Provisional and Non-Provisional Patent Applications and International Patent Applications are herein incorporated by reference in their entireties: U.S. Provisional Application Serial Nos.: 60/368,548 filed April 1, 2002; 60/336,726 filed December 7, 2001; 60/331,478 filed November 16, 2001; 60/330,835 filed Oct 31, 2001; 60/329,747 filed October 18, 2001; and 60/329,508 filed October 17, 2001; 60/225,628 filed August 15, 2000; 60/227,008 filed August 23, 2000; 60/234,338 filed September 22, 2000; 60/240,806 filed October 17, 2000; 60/250,020 filed November 30, 2000; 60/276,248 filed March 6, 2001; 60/293,499 filed May 25, 2001; 60/296,122 filed June 7,

2001; 60/304,809 filed July, 13 2001; 60/122,388 filed March 2, 1999; 60/124,097 filed March 12, 1999; 60/126,599 filed March 26, 2000; 60/127,598 filed April 2, 1999; 60/130,412 filed April 16, 1999; 60/130,696 filed April 23, 1999; 60/131,278 filed April 27, 1999; 60/131,673 filed April 29, 1999; 60/136,784 filed May 28, 1999; 60/142,659 filed July 6, 1999; 60/145,824 filed July 27, 1999; 60/167,239 filed November 24, 1999; 60/168,624 filed December 3, 1999; 60/171,108 filed December 16, 1999; 60/171,626 filed December 23, 1999; 60/176,015 filed January 14, 2000; and, 60/036,100 filed January 14, 1997 and U.S. Nonprovisional Application Serial Nos.: 09/929,493, filed August 14, 2001; 09/588,947 filed June 8, 2000; 09/589,285 filed June 8, 2000; 09/589,286 filed June 8, 2000; 09/589,287 filed June 8, 2000; 09/589,288 filed June 8, 2000; 09/507,968 filed February 22, 2000; 09/255,794 filed February 23, 1999; and 09/005,874 filed January 12, 1998; and International Patent Application Serial Nos. PCT/US01/25549 filed August 15, 2001; PCT/US00/04336, filed February 22, 2000; and PCT/US96/17957, filed October 25, 1996.

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Name of depositary institution: American Type Culture Collection

Address of depositary institution (*including postal code and country*)

10801 University Boulevard
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United States of America

Date of deposit	Accession Number
October 22, 1996	97768

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AUSTRALIA

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FINLAND

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United States of America

Date of deposit	Accession Number
December 12, 1998	203518

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AUSTRALIA

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FINLAND

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10801 University Boulevard
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United States of America

Date of deposit	Accession Number
January 27, 2000	PTA-1158

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ATCC Deposit No.: PTA-1158

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 253, paragraph 519.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet

Name of depositary institution: American Type Culture Collection

Address of depositary institution (*including postal code and country*)

10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
January 27, 2000	PTA-1159

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*) This information is continued on an additional sheet

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (*if the indications are not for all designated States*)

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indications listed below will be submitted to the international Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

	For receiving Office use only			For International Bureau use only	
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:				
Uma Rivera					
PTO/USPTO - I/P/D Team 1 105-305-3073 (703) 305-3080 FAX	Authorized officer				

ATCC Deposit No. PTA-1159**CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

ATCC Deposit No.: PTA-1159

UNITED KINGDOM

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DENMARK

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SWEDEN

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NETHERLANDS

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 254, paragraph 520.

B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet

Name of depositary institution: American Type Culture Collection

Address of depositary institution (*including postal code and country*)

10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
October 24, 2001	PTA-3794

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*) This information is continued on an additional sheet

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (*if the indications are not for all designated States*)

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indications listed below will be submitted to the international Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

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<input type="checkbox"/> This sheet was received with the international application PCT/US02/32910 - USPD Team 1 10/24/2001 0000 0000 0000 0000		<input type="checkbox"/> This sheet was received by the International Bureau on:			
Authorized officer		Authorized officer			

ATCC Deposit No. PTA-3794**CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

ATCC Deposit No.: PTA-3794

UNITED KINGDOM

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DENMARK

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SWEDEN

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3

NETHERLANDS

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 254, paragraph 520.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet

Name of depositary institution: American Type Culture Collection

Address of depositary institution (*including postal code and country*)
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
October 24, 2001	PTA-3795

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*) This information is continued on an additional sheet

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (*if the indications are not for all designated States*)

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC). Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indications listed below will be submitted to the international Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

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<input checked="" type="checkbox"/> This sheet was received with the international application Elonora Rivera		<input type="checkbox"/> This sheet was received by the International Bureau on:							
PCT Operations - IAP Team 1 Authorized officer (703) 305-3678 (703) 305-3230 (FAX)		Authorized officer							

ATCC Deposit No. PTA-3795**CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

ATCC Deposit No.: PTA-3795

UNITED KINGDOM

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DENMARK

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SWEDEN

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NETHERLANDS

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 415, paragraph 1052.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet

Name of depositary institution: American Type Culture Collection

Address of depositary institution (*including postal code and country*)

10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
October 8, 2001	PTA-3778

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*) This information is continued on an additional sheet

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (*if the indications are not for all designated States*)

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indications listed below will be submitted to the international Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

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<input checked="" type="checkbox"/> This sheet was received with the international application Eniola Rivera PCT Operations - IAPD Team 1		<input type="checkbox"/> This sheet was received by the International Bureau on:						
Authorized officer (703) 305-3678 (703) 305-3230 (FAX)		Authorized officer						

ATCC Deposit No. PTA-3778**CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

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FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

ATCC Deposit No.: PTA-3778

UNITED KINGDOM

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DENMARK

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SWEDEN

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NETHERLANDS

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**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on Page 416, paragraph 1053.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet

Name of depositary institution: American Type Culture Collection

Address of depositary institution (*including postal code and country*)
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
November 16, 2001	PTA-3867

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*) This information is continued on an additional sheet

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (*if the indications are not for all designated States*)

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indications listed below will be submitted to the international Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

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PCT Operations - IAPD Team 1 Authorized officer (703) 305-3678 (703) 305-3230 (FAX)	Authorized officer				

ATCC Deposit No. PTA-3867**CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

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AUSTRALIA

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FINLAND

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ATCC Deposit No.: PTA-3867

UNITED KINGDOM

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DENMARK

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What Is Claimed Is:

1. A method of treating a cancer of the immune system comprising administering to an individual, a therapeutically effective amount of a protein comprising an amino acid sequence that is 95% or more identical to a second amino acid sequence selected from the group consisting of:
 - (a) the amino acid sequence of amino acid residues n to 285 of SEQ ID NO:2, where n is an integer in the range of 2-190;
 - (b) the amino acid sequence of amino acid residues 1 to m of SEQ ID NO:2, where m is an integer in the range of 274 to 284; and
 - (c) the amino acid sequence of amino acid residues n to m of SEQ ID NO:2, where n is an integer in the range of 2-190 and m is an integer in the range of 274-284;wherein the protein is radiolabeled.
2. The method of claim 1 wherein the second amino acid sequence is (a).
3. The method of claim 1 wherein the second amino acid sequence is (b).
4. The method of claim 1 wherein the second amino acid sequence is (c).
5. The method of claim 1 wherein the protein comprises an amino acid sequence that is 100% identical to the second amino acid sequence.
6. The method of claim 5 wherein the second amino acid sequence is (a).
7. The method of claim 5 wherein the second amino acid sequence is (b).
8. The method of claim 5 wherein the second amino acid sequence is (c).

9. The method of claim 1 wherein the protein also comprises a heterologous amino acid sequence.

10. The method of claim 9 wherein the heterologous amino acid sequence is the amino acid sequence of an immunoglobulin Fc domain.

11. The method of claim 1 wherein said protein is radiolabeled with a radioisotope selected from the group consisting of:

- (a) ^{131}I ;
- (b) ^{125}I ;
- (c) ^{121}I ;
- (d) ^{112}In ; and
- (e) $^{99\text{m}}\text{Tc}$.

12. The method of claim 11 wherein the radioisotope is ^{131}I .

13. The method of claim 1 wherein the protein is cytotoxic to Neutrokin-alpha receptor bearing cells.

14. The method of claim 1 wherein the cancer of the immune system is a tumor.

15. The method of claim 14 wherein the tumor is metastatic.

16. A method of treating a leukemia comprising administering to an individual, a therapeutically effective amount of a protein comprising an amino acid sequence that is 95% or more identical to a second amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of amino acid residues n to 285 of SEQ ID NO:2, where n is an integer in the range of 2-190;

(b) the amino acid sequence of amino acid residues 1 to m of SEQ ID NO:2, where m is an integer in the range of 274 to 284; and

(c) the amino acid sequence of amino acid residues n to m of SEQ ID NO:2, where n is an integer in the range of 2-190 and m is an integer in the range of 274-284;

wherein the protein is radiolabeled.

17. The method of claim 16 wherein the second amino acid sequence is (a).

18. The method of claim 16 wherein the second amino acid sequence is (b).

19. The method of claim 16 wherein the second amino acid sequence is (c).

20. The method of claim 16 wherein the protein comprises an amino acid sequence that is 100% identical to the second amino acid sequence.

21. The method of claim 20 wherein the second amino acid sequence is (a).

22. The method of claim 20 wherein the second amino acid sequence is (b).

23. The method of claim 20 wherein the second amino acid sequence is (c).

24. The method of claim 16 wherein the protein also comprises a heterologous amino acid sequence.

25. The method of claim 24 wherein the heterologous amino acid sequence is the amino acid sequence of an immunoglobulin Fc domain.

26. The method of claim 16 wherein said protein is radiolabeled with a radioisotope selected from the group consisting of:

(a) ^{131}I ;

- (b) ^{125}I ;
- (c) ^{121}I ;
- (d) ^{112}In ; and
- (e) $^{99\text{m}}\text{Tc}$.

27. The method of claim 26 wherein the radioisotope is ^{131}I .

28. A method of treating a cancer of the immune system comprising administering to an individual, a therapeutically effective amount of a multimeric Neutrokinin-alpha protein comprising an amino acid sequence consisting of amino acids 134-285 of SEQ ID NO:2, and wherein the protein is radiolabeled.

29. The method of claim 28 wherein the protein also comprises a heterologous amino acid sequence.

30. The method of claim 29 wherein the heterologous amino acid sequence is the amino acid sequence of an immunoglobulin Fc domain.

31. The method of claim 28 wherein said protein is radiolabeled with a radioisotope selected from the group consisting of:

- (a) ^{131}I ;
- (b) ^{125}I ;
- (c) ^{121}I ;
- (d) ^{112}In ; and
- (e) $^{99\text{m}}\text{Tc}$.

32. The method of claim 31 wherein the radioisotope is ^{131}I .

33. The method of claim 28 wherein the cancer of the immune system is a tumor.

34. The method of claim 33 wherein the tumor is metastatic.

35. A method of treating leukemia comprising administering to an individual, a therapeutically effective amount of a multimeric Neutrokine-alpha protein comprising an amino acid sequence consisting of amino acids 134-285 of SEQ ID NO:2 wherein the protein is radiolabeled.

36. The method of claim 35 wherein the protein also comprises a heterologous amino acid sequence.

37. The method of claim 36 wherein the heterologous amino acid sequence is the amino acid sequence of an immunoglobulin Fc domain.

38. The method of claim 35 wherein said protein is radiolabeled with a radioisotope selected from the group consisting of:

- (a) ^{131}I ;
- (b) ^{125}I ;
- (c) ^{121}I ;
- (d) ^{112}In ; and
- (e) $^{99\text{m}}\text{Tc}$.

39. The method of claim 38 wherein the radioisotope is ^{131}I .

40. The method of claim 35 wherein the protein is cytotoxic to Neutrokine-alpha receptor bearing cells.

41. The pML124-MBPss-BLyS plasmid.

42. A host cell transformed with the plasmid of claim 41.

43. The host cell of claim 42 which is an *E. coli* cell.

44. A method of producing neutrokinin-alpha protein comprising:

- (a) culturing the host cell of claim 43 under conditions sufficient to produce the Neutrokinin-alpha protein encoded by the plasmid; and
- (b) recovering said Neutrokinin-alpha protein.

Neutrokinin- α

1 AAATTCAAGGATAACTCTCCTGAGGGGTGAGCCAAGCCCTGCCATGTAGTGCACGCAGGAC 60

61 ATCAACAAACACAGATAACAGGAAATGATCCATTCCCTGTGGTCACTTATTCTAAAGGCC 120

121 CCAACCTTCAAAGTTCAAGTAGTAGTGATATGGATGACTCCACAGAAAGGGAGCAGTCACGCC 180
1 M D D S T E R E Q S R L 12

181 TTACTTCTTGCTTAAGAAAAGAGAAGAAAATGAAACTGAAGGAGTGTGTTCCATCCTCC 240
13 T S C L K K R E E M K L K E C V S I L P 32
CD-I

241 CACGGAAGGAAAGCCCTCTGTCCGATCCCTCAAAGACGGAAAGCTGCTGGCTGCAACCT 300
33 R K E S P S V R S S K D G K L L A A T L 52
CD-I

301 TGCTGCTGGCACTGCTGTCTTGCTGCCTCACGGTGGTGTCTTCTACCAAGGTGGCCGCC 360
53 L L A L L S C C L T V V S F Y Q V A A L 72

361 TGCAAGGGACCTGGCCAGCCTCCGGGCAGAGCTGCAGGGCCACCA CGCGGAGAAGCTGC 420
73 Q G D L A S L R A E L Q G H H A E K L P 92
CD-II

421 CAGCAGGAGCAGGAGCCCCAAGGCCGGCTGGAGGAAGCTCCAGCTGTCACCGCCGGAC 480
93 A G A G A P K A G L E E A P A V T A G L 112
CD-III

#

481 TGAAAATCTTGAACCACCAAGCTCCAGGAGAAGGCAACTCCAGTCAGAACAGCAGAAATA 540
113 K I F E P P A P G E G N S S Q N S R N K 132

541 AGCGTGCCGTTCAAGGGTCCAGAAGAACAGTCACTCAAGACTGCTTGCAACTGATTGCAG 600
133 R A V Q G P E E T V T Q D C L Q L I A D 152
CD-IV

FIG. 1A

Neutrokinin- α

601	ACAGTGAAACACCAACTATACAAAAAGGATCTTACACATTGTTCCATGGCTCTCAGCT	660
153	S E T P T I Q K G S Y T F V P W L L S E	172
		CD-V
661	TTAAAAGGGGAAGTGCCTAGAAGAAAAAGAGAATAAAATATTGGTCAAAGAAACTGGTT	720
173	<u>K R G S A L E E K E N K I L V K E T G Y</u>	192
	CD-V	CD-VI
721	ACTTTTTATATATGGTCAGGTTTATATACTGATAAGACCTACGCCATGGGACATCTAA	780
193	<u>F F I Y G Q V L Y T D K T Y A M G H L I</u>	212
	CD-VI	CD-VII
781	TTCAGAGGAAGAAGGTCCATGTCTTGGGGATGAATTGAGTCTGGTACTTTGTTCGAT	840
213	<u>Q R K K V H V F G D E L S L V T L F R C</u>	232
	CD-VII	CD-VIII
841	GTATTCAAAATATGCCTGAAACACTACCCAATAATTCTGCTATTAGCTGGCATTGCAA	900
233	<u>I Q N M P E T L P N N S C Y S A G I A K</u>	252
	CD-VIII	CD-IX
901	AACTGGAAGAAGGAGATGAACTCCAACTTGCAATACCAAGAGAAAATGCACAAATATCAC	960
253	<u>L E E G D E L O L A I P R E N A Q I S L</u>	272
	CD-X	
961	TGGATGGAGATGTCACATTTGGTGCATTGAAACTGCTGTGACCTACTTACACCAGT	1020
273	<u>D G D V T F F G A L K L L</u>	285
	CD-XI	
1021	CTGTAGCTATTTCTCCCTTCTGTACCTCTAAGAAGAAAGAATCTAACTGAAAATA	1080
1081	CCAAAAAAA 1100	

FIG.1B

1	M S T E S M I R D V E I	10	- - - - -	20	- - - - -	30	A E E A
1	M - - - - -	-	- - - - -	-	- - - - -	T P P E R L	TNFalpha
1	M G A - - - - -	-	- - - - -	-	- - - - -	-	TNFbeta
1	M Q Q P F N Y P Y P Q I Y W - V D S S A S S P W A P P G T V	-	-	-	-	-	LTbeta
1	M D D S T E R E Q S R I T S C L K K R E E M K L K E C V S I	-	-	-	-	-	FasLigand
1	M D D S T E R E Q S R I T S C L K K R E E M K L K E C V S I	-	-	-	-	-	Neutrokinne alpha
1	M D D S T E R E Q S R I T S C L K K R E E M K L K E C V S I	-	-	-	-	-	Neutrokinne alphaSV
17	L P K K T G G P Q - - G S R R	40	- - - - -	50	- - - - -	60	- - - - -
8	F - - - - -	-	- - - - -	-	- - - - -	-	TNFalpha
4	- - - L G L E G R G G	-	- - - - -	-	- - - - -	-	TNFbeta
30	L P C P T S V P R R P G Q R R P P P P P P L P P P P P	-	-	-	-	-	LTbeta
31	L P R K E S P S V R S S K D - - G K L L A A T L L A L L	-	-	-	-	-	FasLigand
31	L P R K E S P S V R S S K D - - G K L L A A T L L A L L	-	-	-	-	-	Neutrokinne alpha
31	L P R K E S P S V R S S K D - - G K L L A A T L L A L L	-	-	-	-	-	Neutrokinne alphaSV
30	- - - - - I P R V R G T T L H I L L G L L V L L P	70	- - - - -	80	- - - - -	90	C I F I S I F S
9	- - - - - R L Q G R G S L I L A V A G A T S I V T	-	-	-	-	-	TNFalpha
12	- - - - - P P L P I P P L K R G N H S T G I C I V N F F M	-	-	-	-	-	TNFbeta
60	P P P I P P L P I Q G D L A S I R A E I Q G H H	-	-	-	-	-	LTbeta
58	S C C I T V V S F Y Q V A A L Q G D L A S I R A E I Q G H H	-	-	-	-	-	FasLigand
58	S C C I T V V S F Y Q V A A L Q G D L A S I R A E I Q G H H	-	-	-	-	-	Neutrokinne alpha
							Neutrokinne alphaSV

FIG. 2A

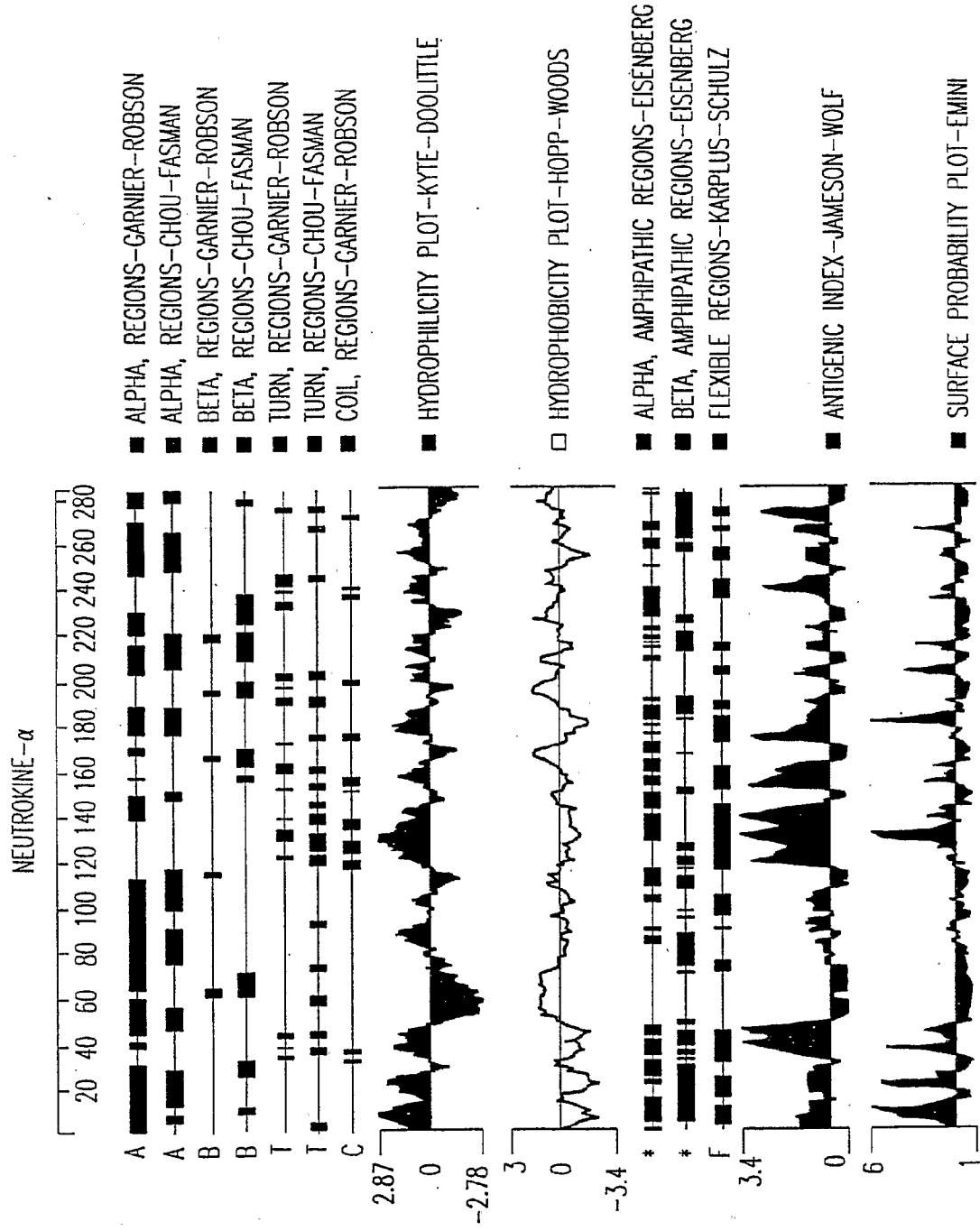
114	A N G V E E R D N - Q L V V P S E G L Y L I Y S Q V L F K G	190	210	TNFalpha
89	Q D G F S L S N N - S L L V P T S G I Y F V Y S Q V V F S G			TNFbeta
114	T S G T Q F S D A E G L A L P Q D G L Y Y L Y C L V G Y R G			LTbeta
172	- S G V K Y K K G - G L V I N E T G L Y F V Y S K V Y F R G			FasLigand
174	R G S A L E E K E N K I L V K E T G Y F F I Y G Q V L Y T D			Neutrokinne alpha
155	R G S A L E E K E N K I L V K E T G Y F F I Y G Q V L Y T D			Neutrokinne alphaSV
143	Q G C P - - - S T H V L L T H T I S R I A V S Y Q T K	220	240	TNFalpha
118	K A Y S P - - K A T S S P I Y L A H E V Q L F S S Q Y P F H			TNFbeta
144	R A P P G G D P Q G R S V T L R S S L Y R A G G A Y G P G			LTbeta
200	Q S C N - - - - N I P L S H K V Y M R N S K Y P Q D			FasLigand
204	K T Y A M G - - - - H L I Q R K K V H V F G D E L S -			Neutrokinne alpha
185	K T Y A M G - - - - H L I Q R K K V H V F G D E L S -			Neutrokinne alphaSV
167	V N - L L S A I K S P C Q R E T P E - - G A E A K P W Y E	250	270	TNFalpha
146	V P - L L S S Q K M V Y P - - - - G L Q E P W L H			TNFbeta
174	T P E L L L E G A E T V T P V L D P A R R Q G Y G P L W Y T			LTbeta
222	L V - M M E G K M M S Y C - - - - T T G Q M W A R			FasLigand
226	L V T L F R C I Q N M P E T L P N - - - - - - - - N			Neutrokinne alpha
207	L V T L F R C I Q N M P E T L P N - - - - - - - - N			Neutrokinne alphaSV

FIG. 2C

		280	290	300	
193	P I Y L G G V F Q L E K G D R L S A E I N R P D Y L D F A E				TNFalpha
166	S M Y H G A A F Q L T Q G D Q L S T H T D G I P H L V L S P				TNFbeta
204	S V G F G G L V Q L R R G E R V Y Y N I S H P D M V D F A R				LTbeta
242	S S Y L G A V F N L T S A D H L T V N V S E L S L V N F E E				FasLigand
244	S C Y S A G I A K L E E G D E L Q L A I P R E N A Q I S L D				Neutrokinne alpha
225	S C Y S A G I A K L E E G D E L Q L A I P R E N A Q I S L D				Neutrokinne alphaSV
		310			
223	S G Q V Y F G I I A L				TNFalpha
196	S - T V F F G A F A L				TNFbeta
234	- G K T F F G A V M V G				LTbeta
272	S - Q T F F G L Y K L				FasLigand
274	G D V T F F G A L K L L				Neutrokinne alpha
255	G D V T F F G A L K L L				Neutrokinne alphaSV

FIG. 2D

FIG. 3



	1	50
HSOAD55RA GGNTAACTCT CCTGAGGGGT GAGCCAAGCC CTGCCATGTA	
HNEDU15X	...AAATTCA GGATAACTCT CCTGAGGGGT GAGCCAAGCC CTGCCATGTA	
HSLAH84R	..AATTGGCA NAGNAAACTG GTTACTTTT TATATATGGT CAGGTTTAT	
HLTBM08R	AATTGGCAC GAGCAAGGCC GGCCTGGAGG AAGCTCCAGC TGTCACCGCG	
	51	100
HSOAD55R	GTGCACGCAG GACATCANCA A..ACACANN NNNCAGGAAA TAATCCATT	
HNEDU15X	GTGCACGCAG GACATCAACA A..ACACAGA TAACAGGAAA TGATCCATT	
HSLAH84R	ATACTGATAA GACCTACGCC ATGGGACATC TAGTTCAGAG GAAGAAGGTC	
HLTBM08R	GGACTGAAAAA TCTTGAACC ACCAGCTCCA GGAGAAGGCA ACTCCAGTCA	
	101	150
HSOAD55R	CCTGTGGTCA CTTATTCTAA AGGCCCAAC CTTCAAAGTT CAAGTAGTGA	
HNEDU15X	CCTGTGGTCA CTTATTCTAA AGGCCCAAC CTTCAAAGTT CAAGTAGTGA	
HSLAH84R	CATGTCTTG GGGATGAATT GAGTCTGGTG ACTTTGTTTC GATGTATTCA	
HLTBM08R	GAACAGCAGA AATAAGCGTG CCGTTCAAGGG TCCAGAAGAA ACAGTCACTC	
	151	200
HSOAD55R	TATGGATGAC TCCACAGAAA GGGAGCAGTC ACGCCTTACT TCTTGCCTTA	
HNEDU15X	TATGGATGAC TCCACAGAAA GGGAGCAGTC ACGCCTTACT TCTTGCCTTA	
HSLAH84R	AAATATGCCT GAAACACTAC CCAATAATTCTGCTATTCA GCTGGCATTG	
HLTBM08R	AAGACTGCTT GCAACTGNNT GCAGACAGTG AAACACCAAC TATACAAAAAA	
	201	250
HSOAD55R	AGAAAAGAGA AGAAATGAAA CTGNAAGGAG TGTGTTCCA TCCTCCCACG	
HNEDU15X	AGAAAAGAGA AGAAATGAAA CT.GAAGGAG TGTGTTCCA TCCTCCCACG	
HSLAH84R	CAAAACTGGN AGGAAGGA... .GATGAAC TCCAACTTGC AATACCAGGG	
HLTBM08R	GGCTCCCTTC TGNTGCCACA TTTGGGCCAA GGAATGGAGA GATTCTTCG	
	251	300
HSOAD55R	GAAGGAAAGC CCCTCTNTCC GATCCTCCAA AGACGGAAAG CTGCTGGCTG	
HNEDU15X	GAAGGAAAGC CCCTCTGTCC GATCCTCCAA AGACGGAAAG CTGCTGGCTG	
HSLAH84R	AAAAATGCAC AATTATCACT GGGATGGAGA TGTTCACATT TTTTGGGTGC	
HLTBM08R	TCTGGAAACA TTTGCCAAA CTCTCAGAT ACTCTTNCT CTCTGGGAAT	
	301	350
HSOAD55R	CAACCTTGNT GNTGGCATTG TGTTCTTGCT GNCTCAAGGT GGTGTTNTT.	
HNEDU15X	CAACCTTGCT GCTGGCACTG CTGTCTTGCT GCCTCACGGT GGTGTCTTTC	
HSLAH84R	CATTGAAACT GCTGTGACCT NCTTACANCA NGTGTGTTN GCTATTTNC	
HLTBM08R	CAAAGGAAAAA TCTCTACTTA GATTNACACA TTTGTTCCCA TGGGTNTCTT	
	351	400
HSOAD55R	
HNEDU15X	TACCAGGTGG CCGCCCTGCA AGGGGACCTG GCCAGCCTCC GGGCAGAGCT	
HSLAH84R	CTNCCTNTTC TNTGGTAACC TCTTAGGAAG GAAGGATTCT TAACTGGAA	
HLTBM08R	AAGTTTAAA AGGGGAGTG CCTTAGGAGG AAAAGGGGAT AAATATTGGC	

FIG.4A

	401	450
HSOAD55R
HNEDU15X	GCAGGGCCAC CACGCGGAGA AGCTGCCAGC AGGAGCAGGA GCCCCCAAGG	
HSLAH84R	ATAACCCAAA AAAANNTAA ANGGGTANGN GNNANANGNG GGGNNNGTNN	
HLTBM08R	CAAGGNACTG GTTANTTTNT AAATATGGTC AGGTTTNTAT ANCTGGTAGG	
	451	500
HSOAD55R
HNEDU15X	CCGGCCTGGA GGAAGCTCCA GCTGTCACCG CGGGACTGAA AATCTTGAA	
HSLAH84R	CNNGNNGNNT TTTNGGNNTA TTTNTNNNTN GGGNNNNNGTA AAAATGGGGC	
HLTBM08R	CCTCGCCATG GGCATTNATT CANGGNGAGG NCNNTCTTTT GGGNTGA...	
	501	550
HSOAD55R
HNEDU15X	CCACCAAGCTC CAGGAGAAGG CAACTCCAGT CAGAACAGCA GAAATAAGCG	
HSLAH84R	CNANGGGGGN TTTTTT.....	
HLTBM08R	
	551	600
HSOAD55R
HNEDU15X	TGCCGTTCAAG GGTCCAGAAG AAACAGTCAC TCAAGACTGC TTGCAACTGA	
HSLAH84R	
HLTBM08R	
	601	650
HSOAD55R
HNEDU15X	TTGCAGACAG TGAAACACCA ACTATACAAA AAGGATCTTA CACATTTGTT	
HSLAH84R	
HLTBM08R	
	651	700
HSOAD55R
HNEDU15X	CCATGGCTTC TCAGCTTAA AAGGGGAAGT GCCCTAGAAG AAAAAGAGAA	
HSLAH84R	
HLTBM08R	
	701	750
HSOAD55R
HNEDU15X	AAAATATTG GTCAAAGAAA CTGGTTACTT TTTTATATAT GGTCAGGTTT	
HSLAH84R	
HLTBM08R	
	751	800
HSOAD55R
HNEDU15X	TATATACTGA TAAGACCTAC GCCATGGGAC ATCTAATTCA GAGGAAGAAG	
HSLAH84R	
HLTBM08R	

FIG.4B

	801	850
HSOAD55R
HNEDU15X	GTCCATGTCT TTGGGGATGA ATTGAGTCTG GTGACTTTGT TTGATGTAT	
HSLAH84R
HLTBM08R
	851	900
HSOAD55R
HNEDU15X	TCAAAATATG CCTGAAACAC TACCAATAA TTCCTGCTAT TCAGCTGGCA	
HSLAH84R
HLTBM08R
	901	950
HSOAD55R
HNEDU15X	TTGCAAAACT GGAAGAAGGA GATGAACTCC AACTTGCAAT ACCAAGAGAA	
HSLAH84R
HLTBM08R
	951	1000
HSOAD55R
HNEDU15X	AATGCACAAA TATCACTGGA TGGAGATGTC ACATTTTTG GTGCATTGAA	
HSLAH84R
HLTBM08R
	1001	1050
HSOAD55R
HNEDU15X	ACTGCTGTGA CCTACTTACA CCATGTCTGT AGCTATTTTC CTCCCTTCT	
HSLAH84R
HLTBM08R
	1051	1100
HSOAD55R
HNEDU15X	CTGTACCTCT AAGAAGAAAG AATCTAACTG AAAATACCAA AAAAAAAA	
HSLAH84R
HLTBM08R
	1101	
HSOAD55R	
HNEDU15X	AAAAAA	
HSLAH84R	
HLTBM08R	

FIG.4C

Neutrokinin- α SV

1	ATGGATGACTCCACAGAAAGGGAGCAGTCACGCCTTACTTCTTGCCTTAAGAAAAGAGAA	60
1	M D D S T E R E Q S R L T S C L K K R E	20
61	GAAATGAAACTGAAGGAGTGTGTTCCATCCTCCCACGGAAGGAAAGCCCCTGTCCGA	120
21	E M K L K E C V S I L P R K E S P S V R	40
	CD-I	
121	TCCTCCAAAGACGGAAAGCTGCTGGCTGCAACCTTGCTGCTGGCACTGCTGTCTGCTGC	180
41	S S K D G K L L A A T L L L A L L S C C	60
	CD-I	
181	CTCACGGTGGTGTCTTCTACCAGGTGGCCGCCCTGCAAGGGGACCTGGCCAGCCTCCGG	240
61	L T V V S F Y Q V A A L Q G D L A S L R	80
	CD-II	
241	GCAGAGCTGCAGGCCACCACGCGGAGAAGCTGCCAGCAGGAGCAGGAGCCCCAAGGCC	300
81	A E L Q G H H A E K L P A G A G A P K A	100
	CD-II	
301	GGCCTGGAGGAAGCTCCAGCTGTACCGCGGGACTGAAAATCTTGAACCACAGCTCCA	360
101	G L E E A P A V T A G L K I F E P P A P	120
	CD-III	
	#	
361	GGAGAAGGCAACTCCAGTCAGAACAGCAGAAATAAGCGTGCCTCAGGGTCCAGAAGAA	420
121	G E G N S S Q N S R N K R A V Q G P E E	140
421	ACAGGATCTTACACATTGTTCCATGGCTTCTCAGCTTAAAAGGGGAAGTGCCTAGAA	480
141	T G S Y T F V P W L L S F K R G S A L E	160
	CD-IV	
481	GAAAAAGAGAATAAAATATTGGTCAAAGAAACTGGTTACTTTTTATATATGGTCAGGTT	540
161	E K E N K I L V K E T G Y F F I Y G Q V	180
	CD-IV	
	CD-V	
541	TTATATACTGATAAGACCTACGCCATGGGACATCTAATTCAAGAGGAAGAAGGTCCATGTC	600
181	L Y T D K T Y A M G H L I Q R K K V H V	200
	CD-VI	
	CD-VII	

FIG.5A

Neutrokinin- α SV

601	TTTGGGGATGAATTGAGTCTGGTGA	CTTTGATGTATTCAAAATATGCCTGAAACA	660
201	<u>F G D E L S L V T L F R C I Q N M P E T</u>		220
	CD-VIII	CD-VIII	
661	CTACCCAATAATTCCCTGCTATTCA	GCTGGCATTGCAAAACTGGAAGAAGGAGATGAACTC	720
221	<u>L P N N S C Y S A G I A K L E E G D E L</u>		240
	CD-IX	CD-X	
721	CAACTTGCAATACCAAGAGAAAATGCACAAATATCA	CTGGATGGAGATGTCACATTTTT	780
241	<u>Q L A I P R E N A Q I S L D G D V T F F</u>		260
	CD-X	CD-XI	
781	GGTGCATTGAAACTGCTGTGACCTACTTACACC	ATGTCTGTAGCTATTTCTCCCTTTC	840
261	<u>G A L K L L</u>		266
	CD-XI		
841	TCTGTACCTCTAAGAAGAAGAATCTA	ACTGAAAATACCAAAAAAAA	900
901	AAA	903	

FIG.5B

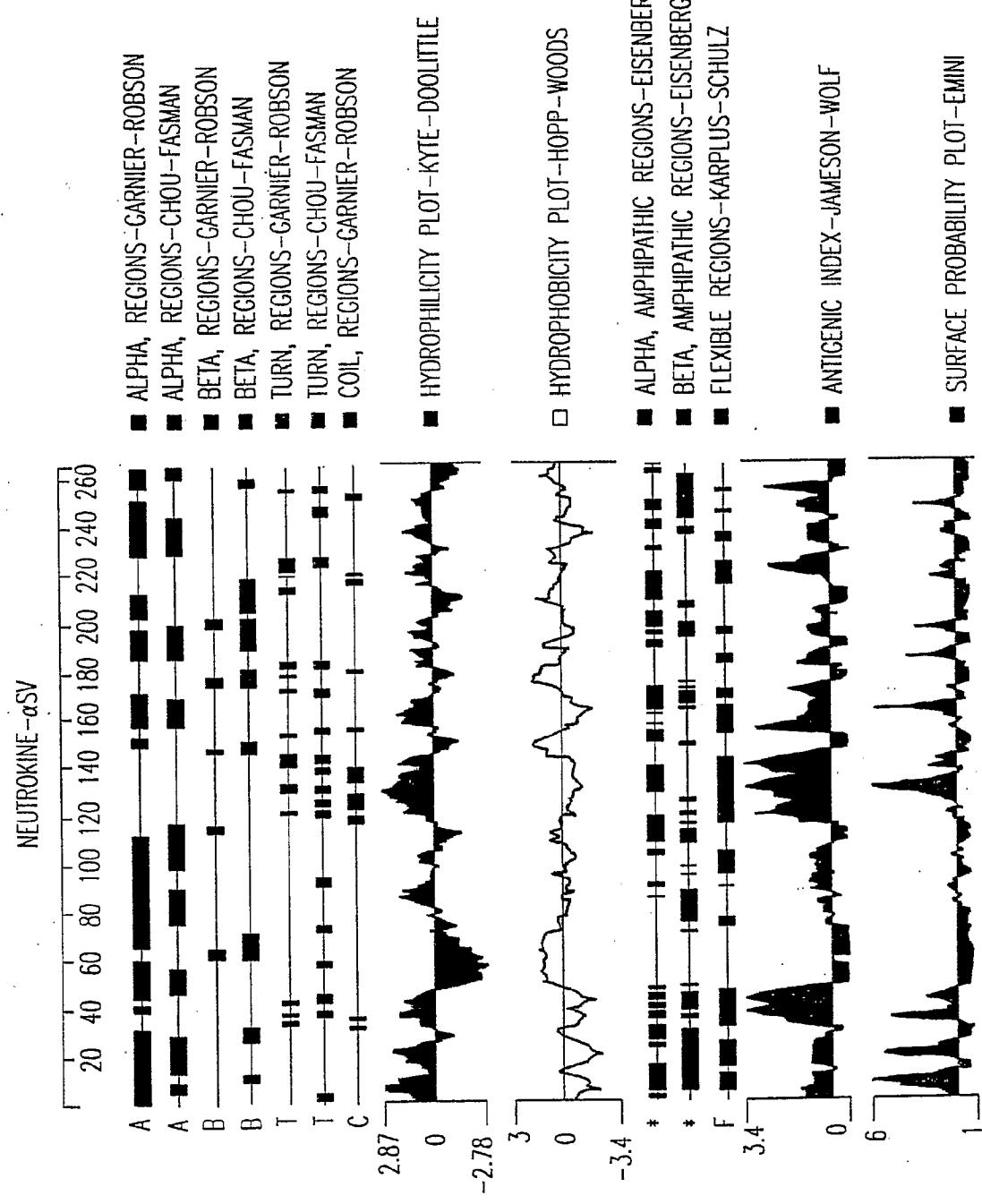


FIG. 6

Transmembrane Region

S K D G K L L A A T L L A L L S C C L T V V S F Y Q V A A L Q G D L A S L R A E 82

L Q G H H A E K L P A G A G A P K A G L E E A P A V T A G L K I F E P P A P G E G 123

<p>A</p> <hr/> <p>N S S Q N S R N K R A V Q G P E E T V T Q D C I Q L I A D S E T P T I Q K G S Y T 164</p> <p>H S V I H I V P I N A I S K - D D S D V I 134</p> <p>April K P V A H V V A N P Q A E G Q - - - - 102</p> <p>TNF K P A A H L I G D P S K Q N S - - - - 76</p> <p>L T α</p>	<p>B</p> <hr/> <p>F P W I L S - - - - F K R G S A E E K E N K I L V K E T G Y F F I Y G Q V L 200</p> <p>E M W Q P A - - - - L R R G R G L Q A Q G Y G V R I Q D A G V Y L L Y S Q V L 170</p> <p>- L Q W I N R R A N A L L A N G V E L R D - N Q L V V P S E G L Y L I Y S Q V L 139</p> <p>- L L W R A N T D R A F L Q D G F S L S N - N S L L V P T S G I Y F V Y S Q V V 114</p>	<p>C</p> <hr/> <p>F Y P W I L S - - - - F K R G S A E E K E N K I L V K E T G Y F F I Y G Q V L 200</p> <p>E M W Q P A - - - - L R R G R G L Q A Q G Y G V R I Q D A G V Y L L Y S Q V L 170</p> <p>- L Q W I N R R A N A L L A N G V E L R D - N Q L V V P S E G L Y L I Y S Q V L 139</p> <p>- L L W R A N T D R A F L Q D G F S L S N - N S L L V P T S G I Y F V Y S Q V V 114</p>
<p>A'</p> <hr/> <p>F Y P W I L S - - - - F K R G S A E E K E N K I L V K E T G Y F F I Y G Q V L 200</p> <p>E M W Q P A - - - - L R R G R G L Q A Q G Y G V R I Q D A G V Y L L Y S Q V L 170</p> <p>- L Q W I N R R A N A L L A N G V E L R D - N Q L V V P S E G L Y L I Y S Q V L 139</p> <p>- L L W R A N T D R A F L Q D G F S L S N - N S L L V P T S G I Y F V Y S Q V V 114</p>	<p>B'</p> <hr/> <p>F Y P W I L S - - - - F K R G S A E E K E N K I L V K E T G Y F F I Y G Q V L 200</p> <p>E M W Q P A - - - - L R R G R G L Q A Q G Y G V R I Q D A G V Y L L Y S Q V L 170</p> <p>- L Q W I N R R A N A L L A N G V E L R D - N Q L V V P S E G L Y L I Y S Q V L 139</p> <p>- L L W R A N T D R A F L Q D G F S L S N - N S L L V P T S G I Y F V Y S Q V V 114</p>	<p>C</p> <hr/> <p>F Y P W I L S - - - - F K R G S A E E K E N K I L V K E T G Y F F I Y G Q V L 200</p> <p>E M W Q P A - - - - L R R G R G L Q A Q G Y G V R I Q D A G V Y L L Y S Q V L 170</p> <p>- L Q W I N R R A N A L L A N G V E L R D - N Q L V V P S E G L Y L I Y S Q V L 139</p> <p>- L L W R A N T D R A F L Q D G F S L S N - N S L L V P T S G I Y F V Y S Q V V 114</p>
<p>D</p> <hr/> <p>Y T D K T Y - - - - A M G H L I Q R K K V H V F G D E L S L V T L F R C I Q N M P 237</p> <p>F Q D V I F - - - - T M G Q V V S R E - - - - G Q G R Q E T L F R G I R S M P 201</p> <p>F K G Q G C P - - - - S T H V L I T H T I S R I A V S Y Q T K V N L L S A I K S P 176</p> <p>F S G K A Y S P K A I S S P L Y L A H E V Q L F S S Q Y P F H V P L L S S Q K M V 155</p>	<p>E</p> <hr/> <p>Y T D K T Y - - - - A M G H L I Q R K K V H V F G D E L S L V T L F R C I Q N M P 237</p> <p>F Q D V I F - - - - T M G Q V V S R E - - - - G Q G R Q E T L F R G I R S M P 201</p> <p>F K G Q G C P - - - - S T H V L I T H T I S R I A V S Y Q T K V N L L S A I K S P 176</p> <p>F S G K A Y S P K A I S S P L Y L A H E V Q L F S S Q Y P F H V P L L S S Q K M V 155</p>	<p>FIG. 7A-1</p>

F		G		H	
E	- T L P	- - - - -	N N S C Y S A G I A K	E E G D E L Q L A I P R E N A	268
S H P D R A	- - - - -	- Y N S C Y S A G V F H	G G D I H Q I S V I I P R A R A	234	
C Q R E T P	- - - - -	P W Y E P I Y L G G V F Q L E	G Q D R L S A E I N R P D Y	217	
Y P	- - - - -	P W L H S M Y H G A A F Q L T	K Q D P Q L S T H T D G I P H	190	
G		G		G	
E	- T L P	- - - - -	N N S C Y S A G I A K	E E G D E L Q L A I P R E N A	285
S H P D R A	- - - - -	- Y N S C Y S A G V F H	G G D I H Q I S V I I P R A R A	250	
C Q R E T P	- - - - -	P W Y E P I Y L G G V F Q L E	G Q D R L S A E I N R P D Y	233	
Y P	- - - - -	P W L H S M Y H G A A F Q L T	K Q D P Q L S T H T D G I P H	205	

FIG. 7A-2

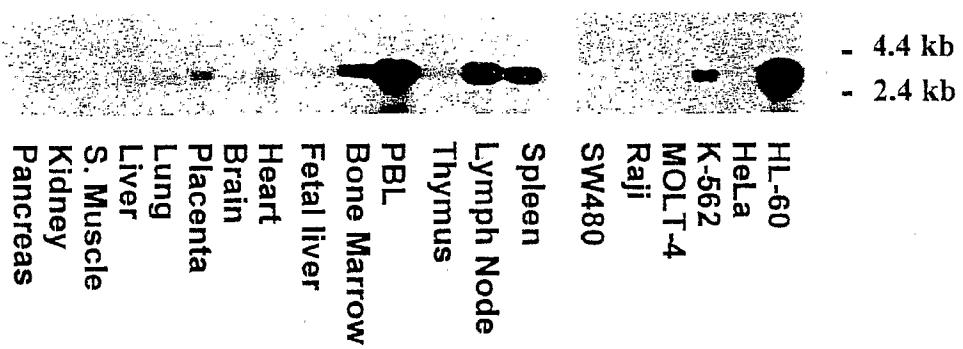


FIG. 7B

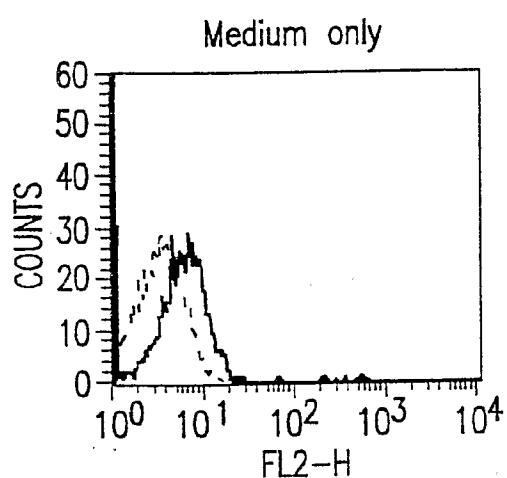


FIG.8A

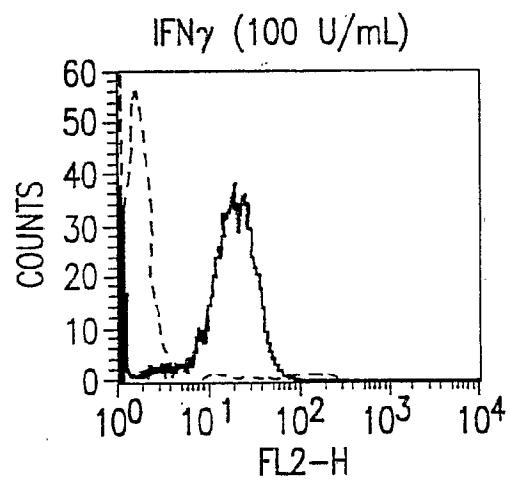


FIG.8B

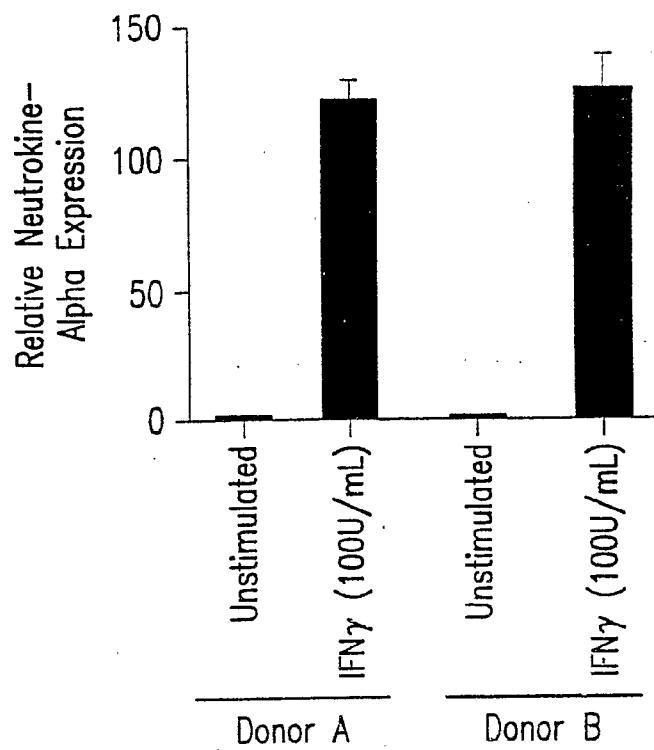


FIG.8C

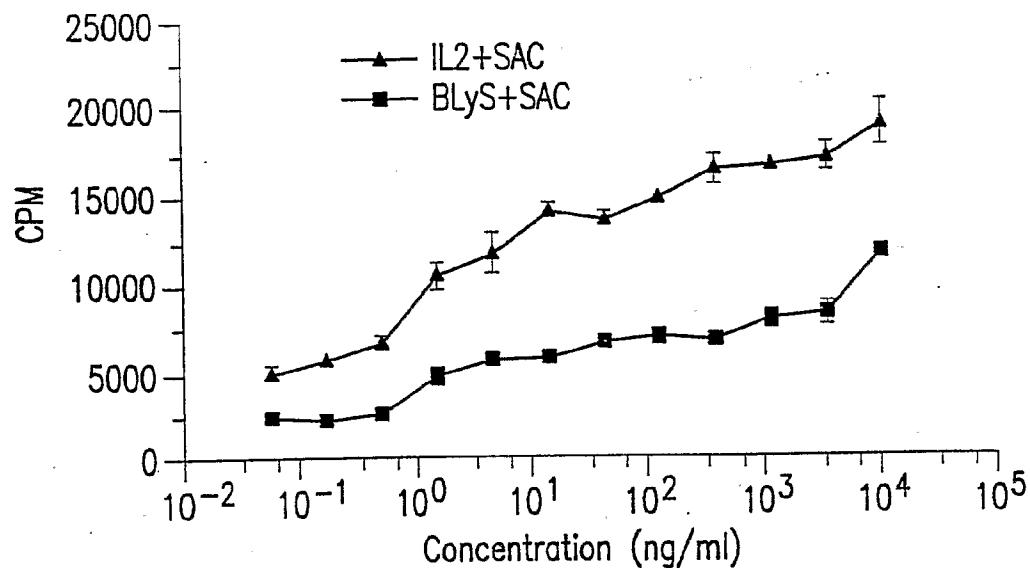


FIG. 9A

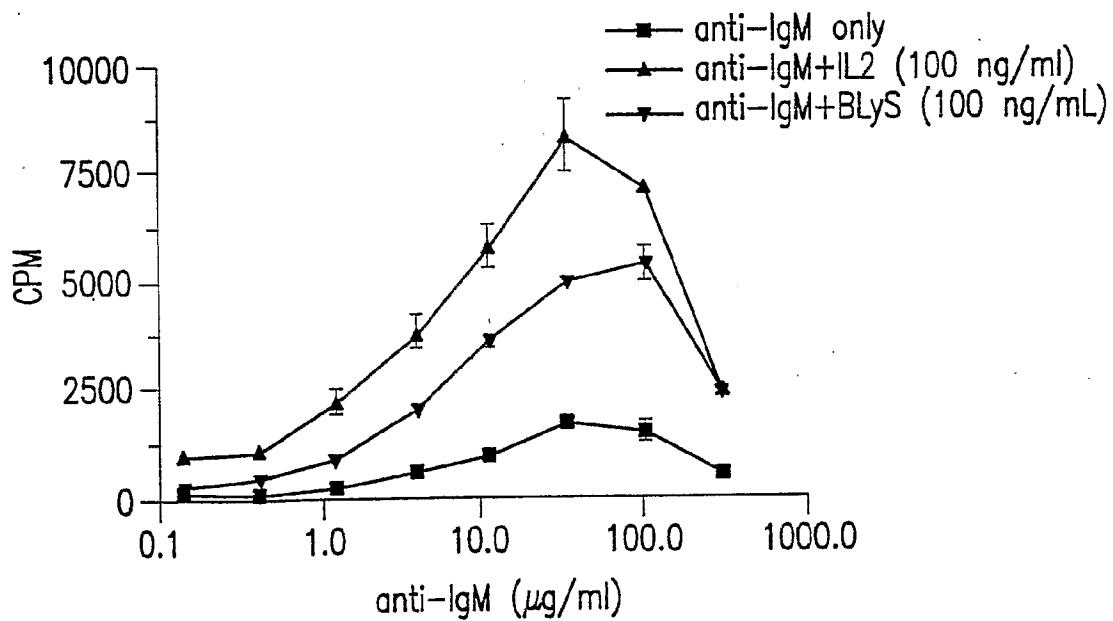


FIG. 9B

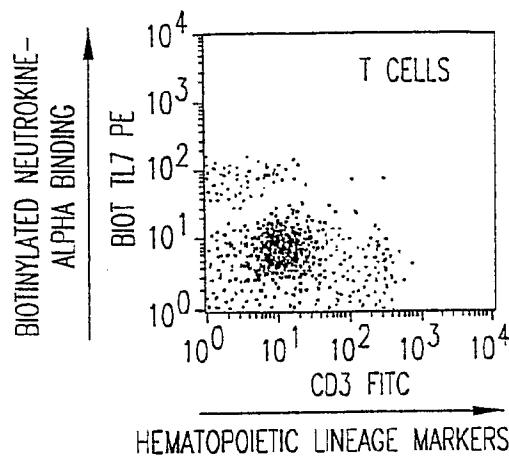


FIG.10A

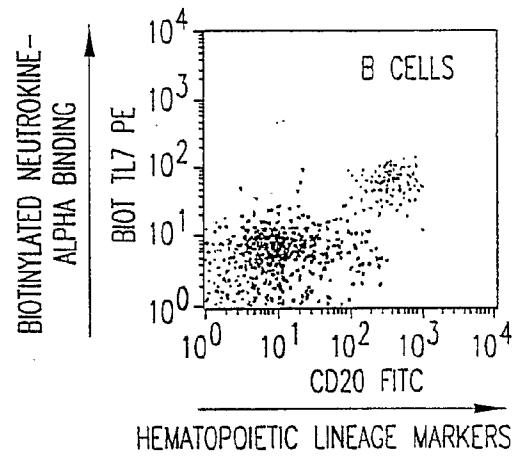


FIG.10B

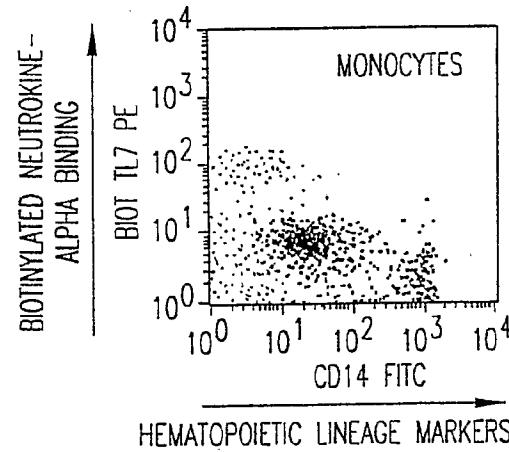


FIG.10C

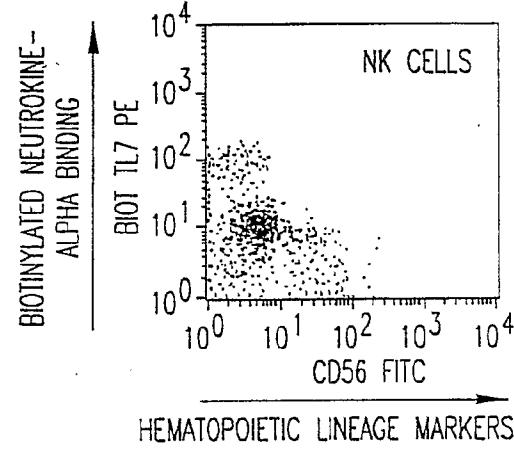


FIG.10D

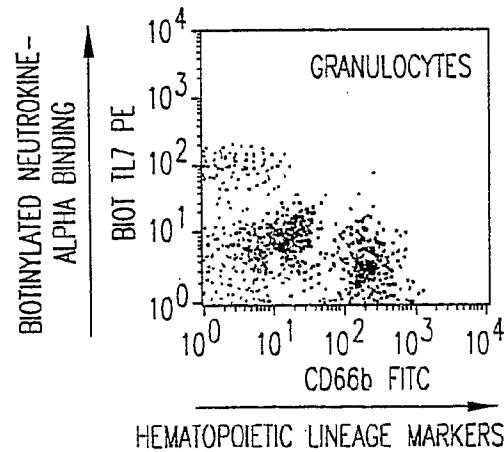


FIG.10E

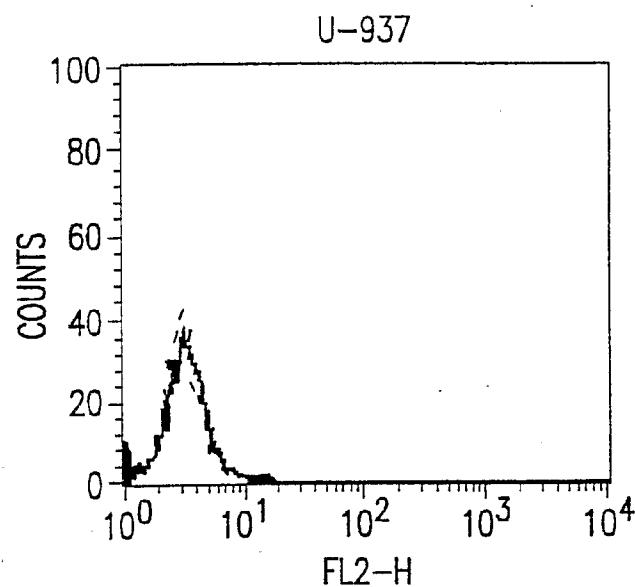


FIG.10F

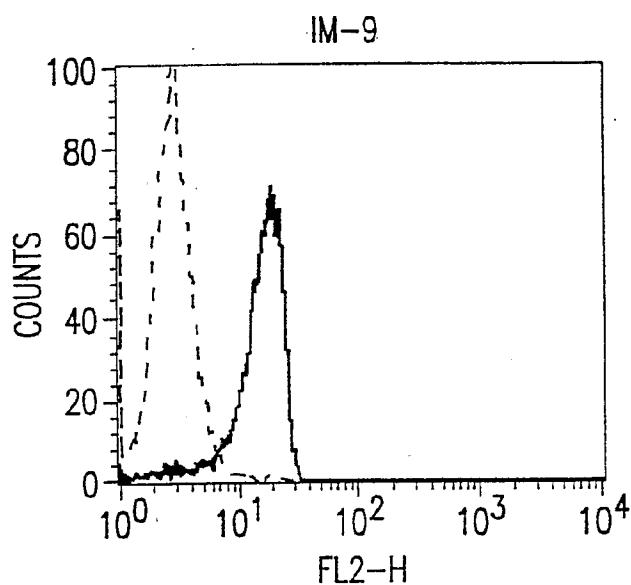


FIG.10G

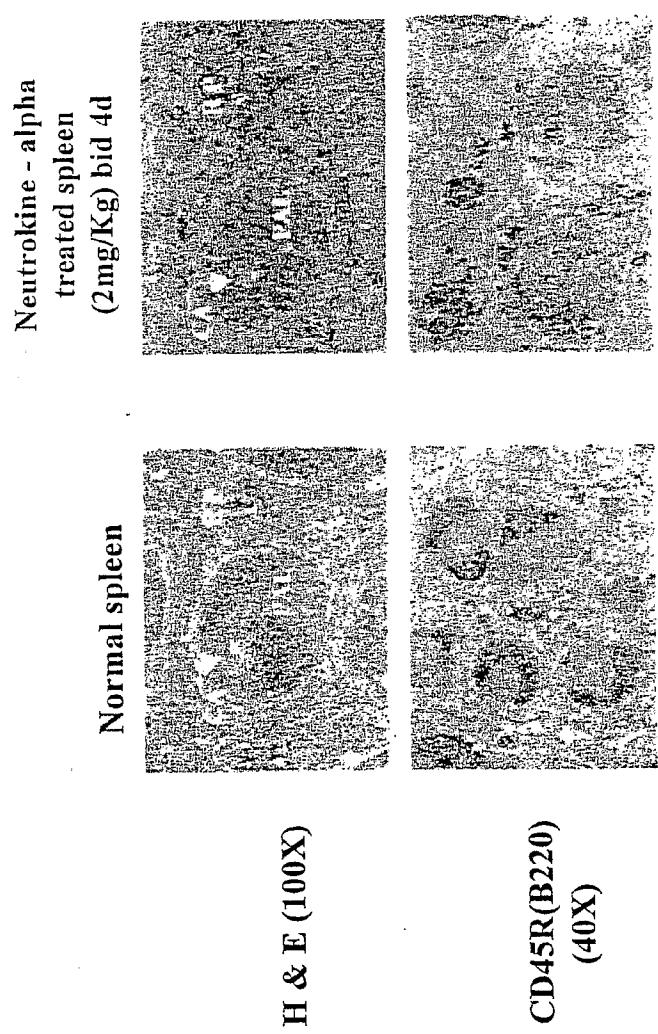


FIG. 11A

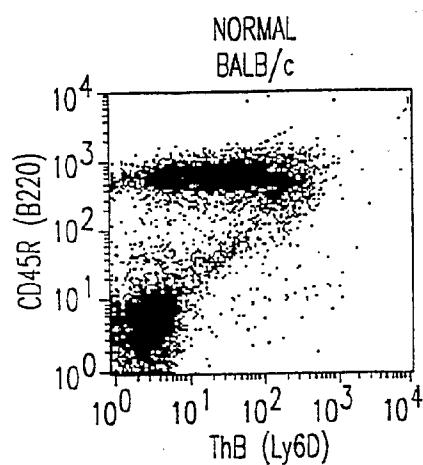


FIG. 11B

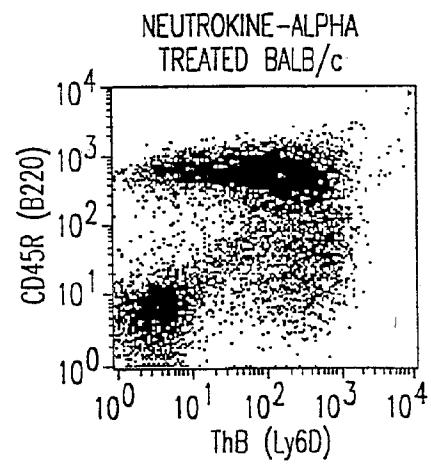


FIG. 11C

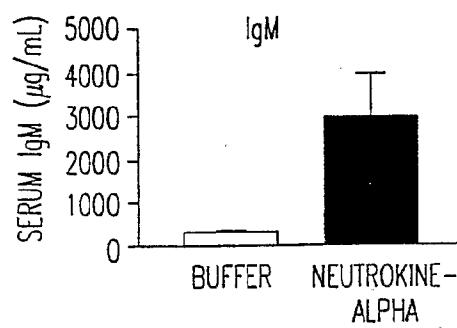


FIG. 11D

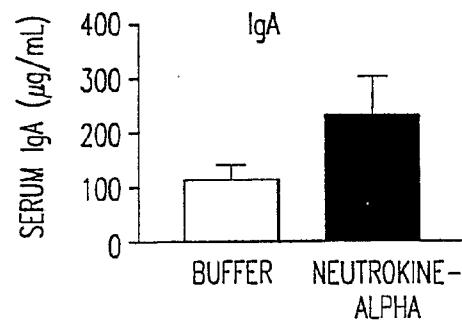


FIG. 11E

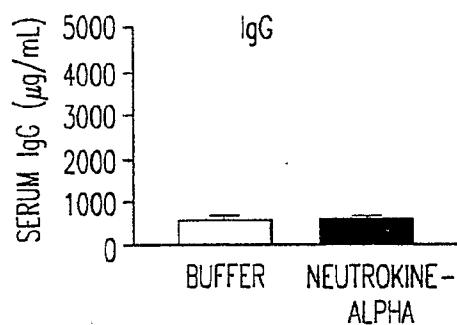


FIG. 11F

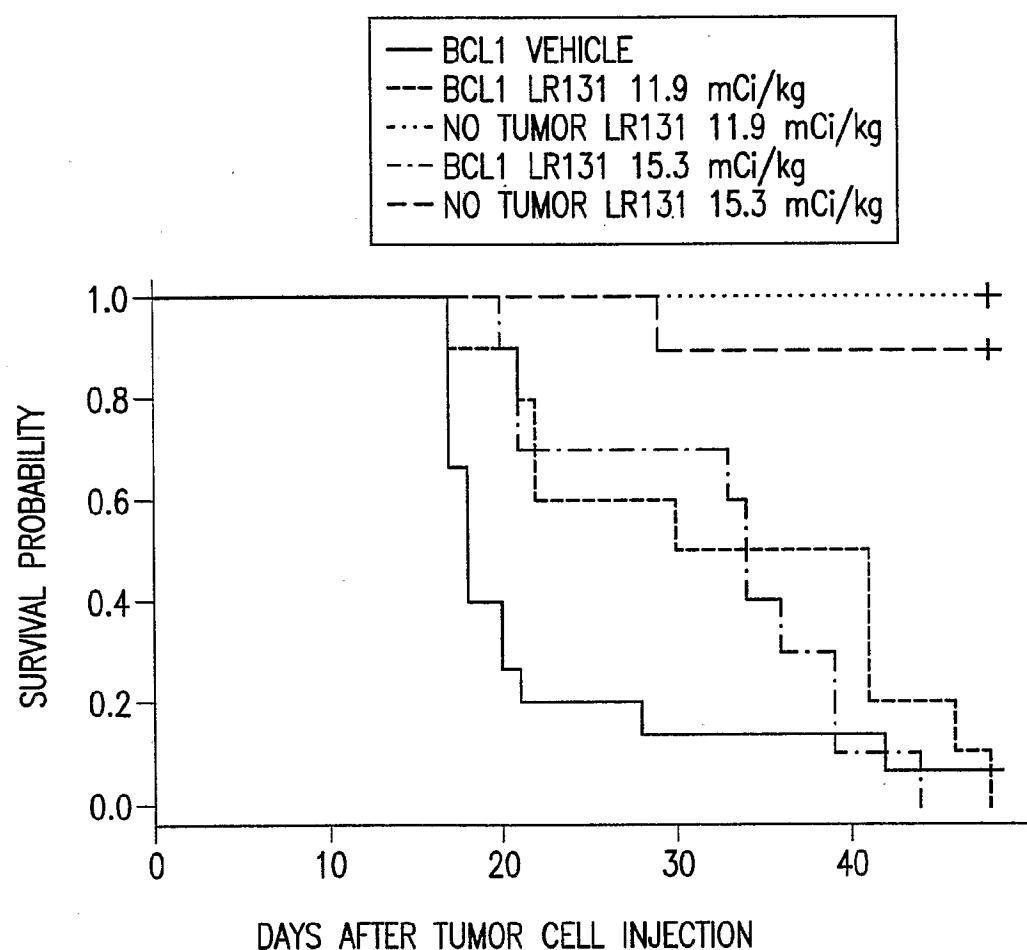


FIG.12

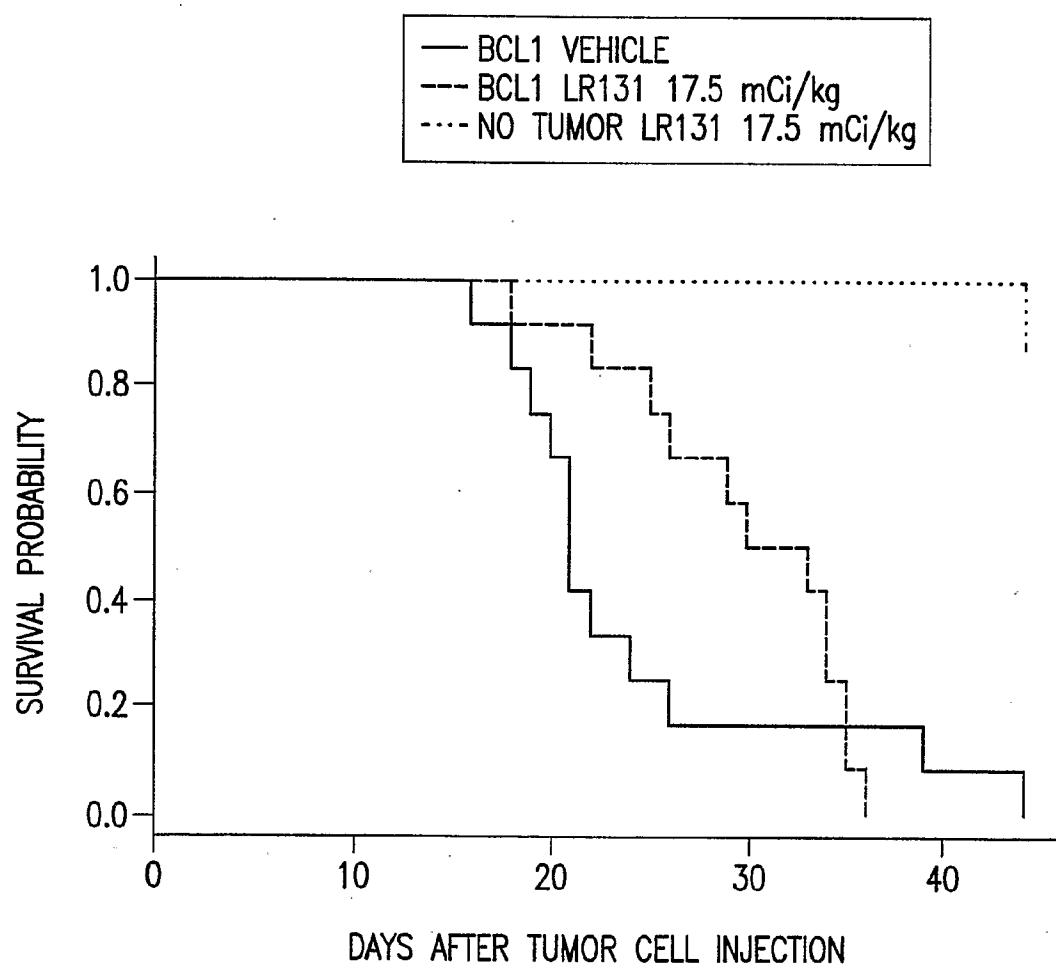


FIG.13

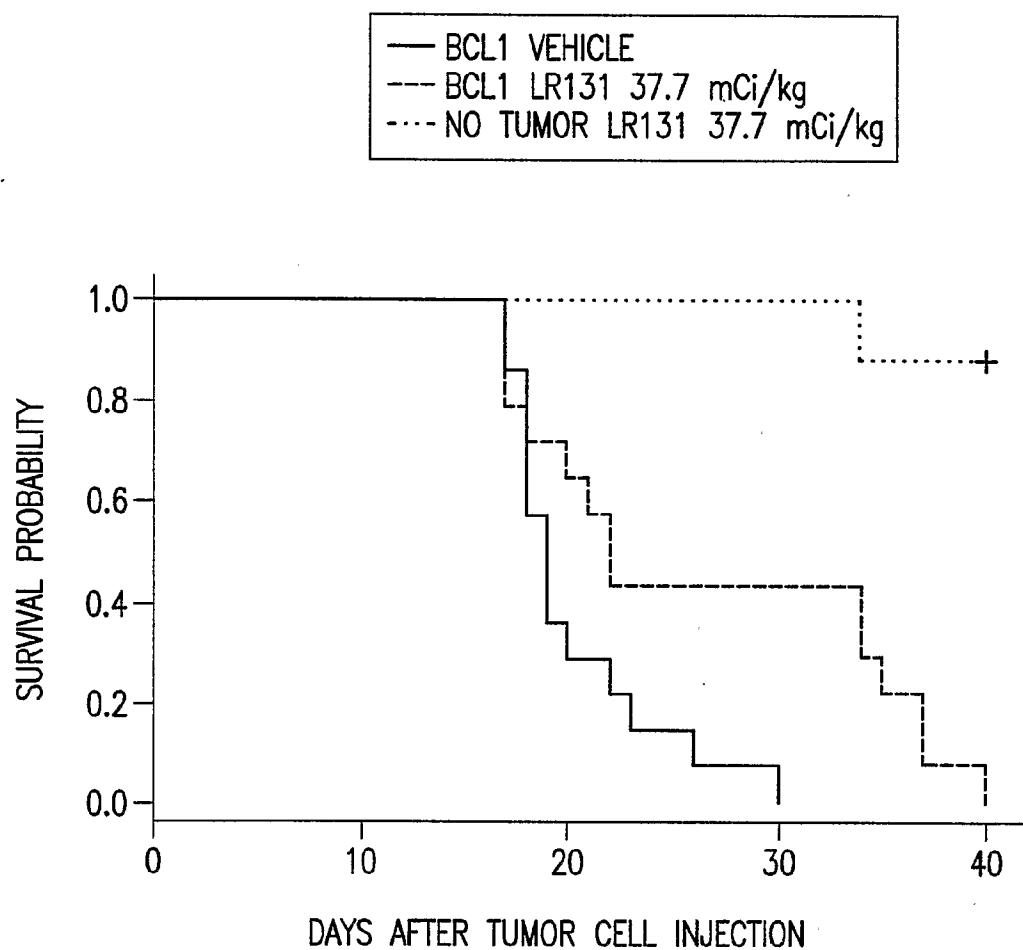


FIG.14

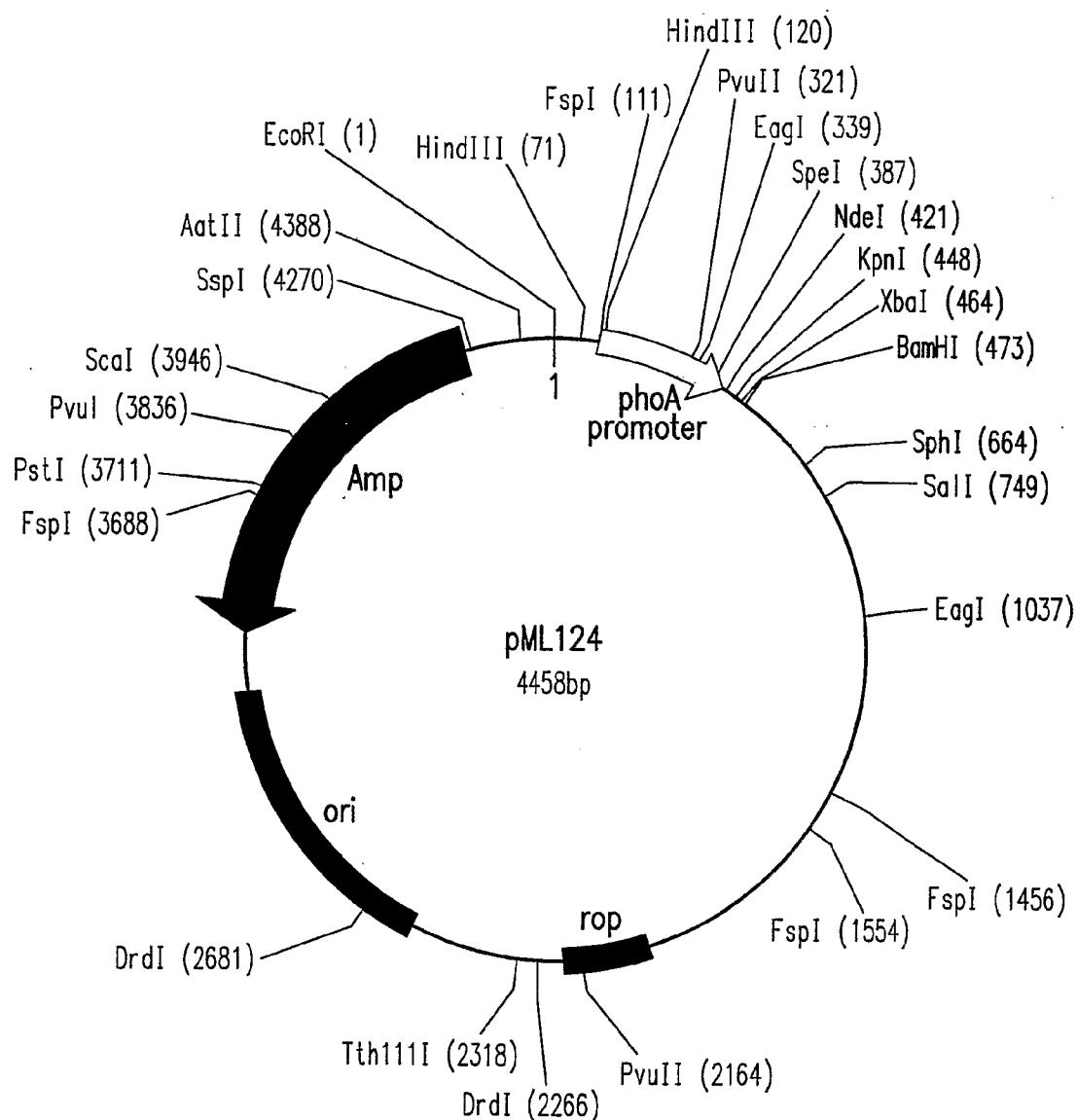


FIG.15

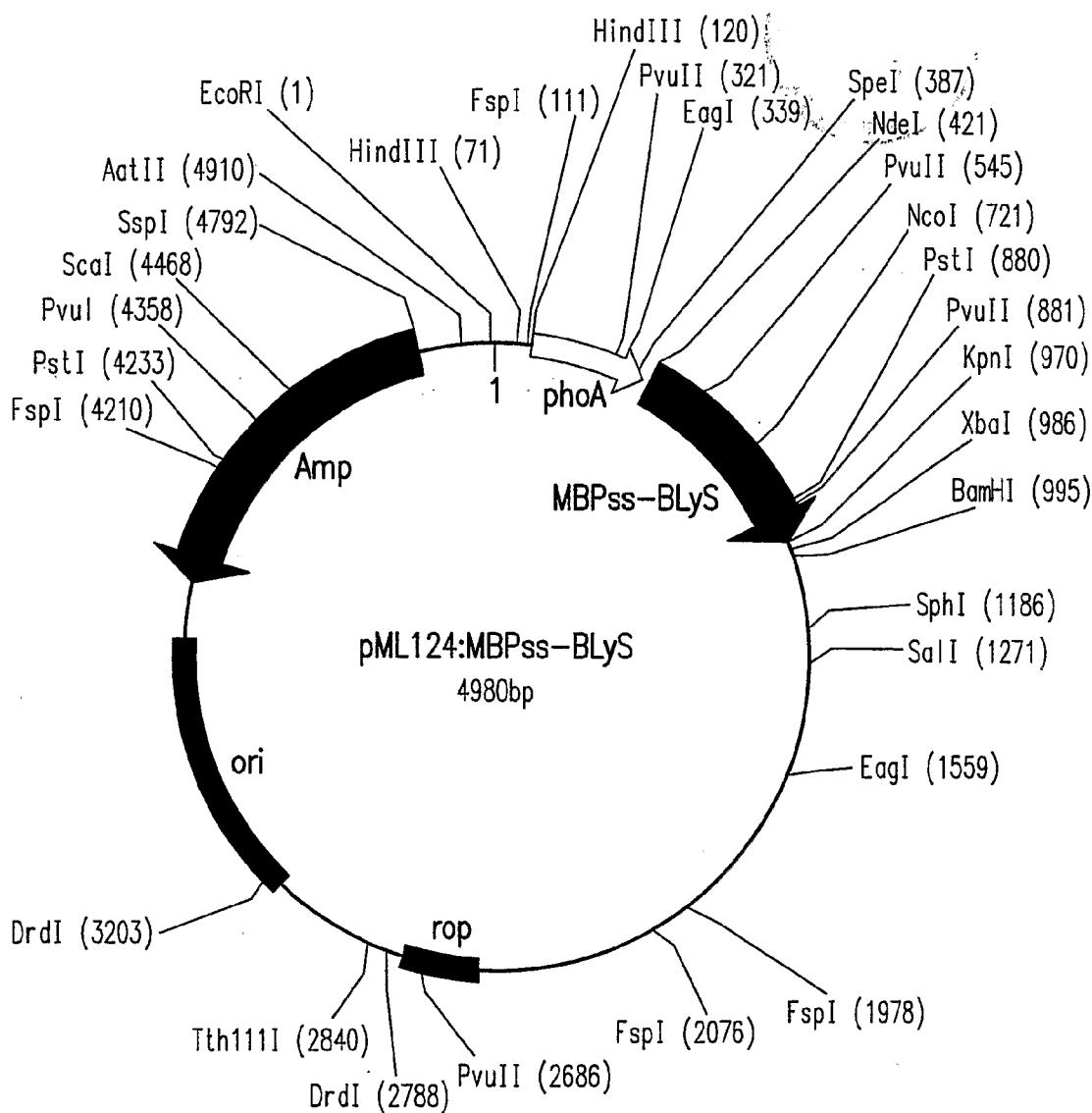


FIG.16

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<151> 2001-12-07

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ccaaccttca aagttcaagt agtgat atg gat gac tcc aca gaa agg gag cag 173
Met Asp Asp Ser Thr Glu Arg Glu Gln

1

5

tca cgc ctt act tct tgc ctt aag aaa aga gaa gaa atg aaa ctg aag 221
Ser Arg Leu Thr Ser Cys Leu Lys Arg Glu Glu Met Lys Leu Lys
10 15 20 25

gag tgt gtt tcc atc ctc cca cgg aag gaa agc ccc tct gtc cga tcc 269
Glu Cys Val Ser Ile Leu Pro Arg Lys Glu Ser Pro Ser Val Arg Ser
30 35 40

tcc aaa gac gga aag ctg ctg gct gca acc ttg ctg ctg gca ctg ctg 317

Ser Lys Asp Gly Lys Leu Leu Ala Ala Thr Leu Leu Ala Leu Leu			
45	50	55	
tct tgc tgc ctc acg gtg gtg tct ttc tac cag gtg gcc gcc ctg caa			365
Ser Cys Cys Leu Thr Val Val Ser Phe Tyr Gln Val Ala Ala Leu Gln			
60	65	70	
ggg gac ctg gcc agc ctc cgg gca gag ctg cag ggc cac cac gcg gag			413
Gly Asp Leu Ala Ser Leu Arg Ala Glu Leu Gln Gly His His Ala Glu			
75	80	85	
aag ctg cca gca gga gca gga gcc ccc aag gcc ggc ctg gag gaa gct			461
Lys Leu Pro Ala Gly Ala Pro Lys Ala Gly Leu Glu Glu Ala			
90	95	100	105
cca gct gtc acc gcg gga ctg aaa atc ttt gaa cca cca gct cca gga			509
Pro Ala Val Thr Ala Gly Leu Lys Ile Phe Glu Pro Pro Ala Pro Gly			
110	115	120	
gaa ggc aac tcc agt cag aac agc aga aat aag cgt gcc gtt cag ggt			557
Glu Gly Asn Ser Ser Gln Asn Ser Arg Asn Lys Arg Ala Val Gln Gly			
125	130	135	
cca gaa gaa aca gtc act caa gac tgc ttg caa ctg att gca gac agt			605
Pro Glu Glu Thr Val Thr Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser			
140	145	150	
gaa aca cca act ata caa aaa gga tct tac aca ttt gtt cca tgg ctt			653
Glu Thr Pro Thr Ile Gln Lys Gly Ser Tyr Thr Phe Val Pro Trp Leu			
155	160	165	
ctc agc ttt aaa agg gga agt gcc cta gaa gaa aaa gag aat aaa ata			701
Leu Ser Phe Lys Arg Gly Ser Ala Leu Glu Lys Glu Asn Lys Ile			
170	175	180	185
ttg gtc aaa gaa act ggt tac ttt ata tat ggt cag gtt tta tat			749
Leu Val Lys Glu Thr Gly Tyr Phe Phe Ile Tyr Gly Gln Val Leu Tyr			
190	195	200	
act gat aag acc tac gcc atg gga cat cta att cag agg aag aag gtc			797
Thr Asp Lys Thr Tyr Ala Met Gly His Leu Ile Gln Arg Lys Lys Val			
205	210	215	
cat gtc ttt ggg gat gaa ttg agt ctg gtg act ttg ttt cga tgt att			845
His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile			
220	225	230	
caa aat atg cct gaa aca cta ccc aat aat tcc tgc tat tca gct ggc			893
Gln Asn Met Pro Glu Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly			
235	240	245	
att gca aaa ctg gaa gaa gga gat gaa ctc caa ctt gca ata cca aga			941
Ile Ala Lys Leu Glu Glu Gly Asp Glu Leu Gln Leu Ala Ile Pro Arg			
250	255	260	265
gaa aat gca caa ata tca ctg gat gga gat gtc aca ttt ttt ggt gca			989
Glu Asn Ala Gln Ile Ser Leu Asp Gly Asp Val Thr Phe Phe Gly Ala			
270	275	280	

ttg aaa ctg ctg tgacctactt acaccatgtc tgtagctatt ttccctccctt 1041
 Leu Lys Leu Leu
 285
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 20 25 30
 Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly Lys Leu Leu
 35 40 45
 Ala Ala Thr Leu Leu Ala Leu Leu Ser Cys Cys Leu Thr Val Val
 50 55 60
 Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp Leu Ala Ser Leu Arg
 65 70 75 80
 Ala Glu Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Gly Ala Gly
 85 90 95
 Ala Pro Lys Ala Gly Leu Glu Glu Ala Pro Ala Val Thr Ala Gly Leu
 100 105 110
 Lys Ile Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Asn
 115 120 125
 Ser Arg Asn Lys Arg Ala Val Gln Gly Pro Glu Glu Thr Val Thr Gln
 130 135 140
 Asp Cys Leu Gln Leu Ile Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys
 145 150 155 160
 Gly Ser Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser
 165 170 175
 Ala Leu Glu Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr
 180 185 190
 Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met
 195 200 205
 Gly His Leu Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu
 210 215 220
 Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu
 225 230 235 240
 Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly
 245 250 255

Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu
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Leu Ser Leu Phe Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
 35 40 45

Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
 50 55 60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Val Arg Ser Ser
 65 70 75 80

Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro
 85 90 95

Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu
 100 105 110

Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser
 115 120 125

Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly
 130 135 140

Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala
 145 150 155 160

Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro
 165 170 175

Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu
 180 185 190

Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu
 195 200 205

Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly
 210 215 220

Gln Val Tyr Phe Gly Ile Ile Ala Leu
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 Gln Gly Leu Pro Gly Val Gly Leu Thr Pro Ser Ala Ala Gln Thr Ala
 35 40 45
 Arg Gln His Pro Lys Met His Leu Ala His Ser Thr Leu Lys Pro Ala
 50 55 60
 Ala His Leu Ile Gly Asp Pro Ser Lys Gln Asn Ser Leu Leu Trp Arg
 65 70 75 80
 Ala Asn Thr Asp Arg Ala Phe Leu Gln Asp Gly Phe Ser Leu Ser Asn
 85 90 95
 Asn Ser Leu Leu Val Pro Thr Ser Gly Ile Tyr Phe Val Tyr Ser Gln
 100 105 110
 Val Val Phe Ser Gly Lys Ala Tyr Ser Pro Lys Ala Thr Ser Ser Pro
 115 120 125
 Leu Tyr Leu Ala His Glu Val Gln Leu Phe Ser Ser Gln Tyr Pro Phe
 130 135 140
 His Val Pro Leu Leu Ser Ser Gln Lys Met Val Tyr Pro Gly Leu Gln
 145 150 155 160
 Glu Pro Trp Leu His Ser Met Tyr His Gly Ala Ala Phe Gln Leu Thr
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 35 40 45
 Gln Asp Gln Gly Gly Leu Val Thr Glu Thr Ala Asp Pro Gly Ala Gln
 50 55 60
 Ala Gln Gln Gly Leu Gly Phe Gln Lys Leu Pro Glu Glu Pro Glu
 65 70 75 80
 Thr Asp Leu Ser Pro Gly Leu Pro Ala Ala His Leu Ile Gly Ala Pro
 85 90 95
 Leu Lys Gly Gln Gly Leu Gly Trp Glu Thr Thr Lys Glu Gln Ala Phe
 100 105 110
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 115 120 125
 Gln Asp Gly Leu Tyr Tyr Leu Tyr Cys Leu Val Gly Tyr Arg Gly Arg
 130 135 140
 Ala Pro Pro Gly Gly Asp Pro Gln Gly Arg Ser Val Thr Leu Arg
 145 150 155 160
 Ser Ser Leu Tyr Arg Ala Gly Gly Ala Tyr Gly Pro Gly Thr Pro Glu
 165 170 175
 Leu Leu Leu Glu Gly Ala Glu Thr Val Thr Pro Val Leu Asp Pro Ala
 180 185 190
 Arg Arg Gln Gly Tyr Gly Pro Leu Trp Tyr Thr Ser Val Gly Phe Gly
 195 200 205
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 Val Met Val Gly

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 Pro Thr Ser Val Pro Arg Arg Pro Gly Gln Arg Arg Pro Pro Pro Pro
 35 40 45
 Pro Pro Pro Pro Pro Leu Pro Pro Pro Pro Pro Pro Pro Pro Leu Pro

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65	70	75
Leu Cys Leu Leu Val Met Phe Phe Met Val Leu Val Ala Leu Val Gly		
85	90	95
Leu Gly Leu Gly Met Phe Gln Leu Phe His Leu Gln Lys Glu Leu Ala		
100	105	110
Glu Leu Arg Glu Ser Thr Ser Gln Met His Thr Ala Ser Ser Leu Glu		
115	120	125
Lys Gln Ile Gly His Pro Ser Pro Pro Glu Lys Lys Glu Leu Arg		
130	135	140
Lys Val Ala His Leu Thr Gly Lys Ser Asn Ser Arg Ser Met Pro Leu		
145	150	155
Glu Trp Glu Asp Thr Tyr Gly Ile Val Leu Leu Ser Gly Val Lys Tyr		
165	170	175
Lys Lys Gly Gly Leu Val Ile Asn Glu Thr Gly Leu Tyr Phe Val Tyr		
180	185	190
Ser Lys Val Tyr Phe Arg Gly Gln Ser Cys Asn Asn Leu Pro Leu Ser		
195	200	205
His Lys Val Tyr Met Arg Asn Ser Lys Tyr Pro Gln Asp Leu Val Met		
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Met Glu Gly Lys Met Met Ser Tyr Cys Thr Thr Gly Gln Met Trp Ala		
225	230	235
Arg Ser Ser Tyr Leu Gly Ala Val Phe Asn Leu Thr Ser Ala Asp His		
245	250	255
Leu Tyr Val Asn Val Ser Glu Leu Ser Leu Val Asn Phe Glu Glu Ser		
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Gln Thr Phe Phe Gly Leu Tyr Lys Leu		
275	280	

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<223> Description of Combined DNA/RNA Molecule: n equals
a, t, g, or c

<220>

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<222> (3)

<223> n equals a, t, g, or c

<220>
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<222> (58)
<223> n equals a, t, g, or c

<220>
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<223> n equals a, t, g, or c

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aaacacan nnccaggaaat aatccattcc ctgtggtcac ttattctaaa ggcccccaacc 120
ttcaaaagttc aagtagtgat atggatgact ccacagaaag ggagcagtca cgccttactt 180
cttgccttaa gaaaagagaa gaaatgaaac tgnaaggagt gtgtttccat cctccacgg 240
aaggaaagcc cctctntccg atcctccaaa gacggaaagc tgctggctgc aaccttgntg 300
ntggcattgt gttcttgctg nctcaagggtg gtgttntt 338

<210> 8
<211> 509
<212> DNA
<213> Homo sapiens

<220>
<223> Description of Combined DNA/RNA Molecule: n equals
a, t, g, or c

<220>
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<222> (10)
<223> n equals a, t, g, or c

<220>
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<223> n equals a, t, g, or c

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<222> (433)
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<222> (438)..(439)
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<220>
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<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (466)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (469)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (471)..(472)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (474)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (478)..(481)
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<220>
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<220>
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<222> (498)
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<220>
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<222> (504)
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aattcggcan agnaaactgg ttactttttt atatatggtc aggttttata tactgataag 60
acctaagccca tgggacatct agttcagagg aagaaggcctc atgtctttgg ggatgaattg 120
agtctggtga ctttgcgtcg atgtattcaa aatatgcctg aaacactacc caataattcc 180
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tgctattcag ctggcattgc aaaactggna ggaaggagat gaactccaac ttgcaataacc 240
 agggggaaaat gcacaattat cactgggatg gagatgttca catttttgg gtgccattga 300
 aactgctgtg acctncttac ancangtgct gttngctatt ttncctncct nttctntgg 360
 aacctcttag gaaggaagga ttcttaactg ggaaataacc caaaaaaann ttaaanggg 420
 angnngnnana ngnggggnng ttncnngnn gnntttngg nnatntnt nntngggnnn 480
 ngtaaaaatg gggccnangg gggntttt 509

<210> 9
 <211> 497
 <212> DNA
 <213> Homo sapiens

<220>
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 <222> (168)
 <223> n equals a, t, g, or c

<220>
 <221> misc_feature
 <222> (213)
 <223> n equals a, t, g, or c

<220>
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 <222> (288)
 <223> n equals a, t, g, or c

<220>
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 <222> (325)
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<220>
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 <222> (346)
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<220>
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 <222> (406)
 <223> n equals a, t, g, or c

<220>
 <221> misc_feature
 <222> (415)
 <223> n equals a, t, g, or c

<220>
 <221> misc_feature
 <222> (419)
 <223> n equals a, t, g, or c

<220>
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 <223> n equals a, t, g, or c

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<222> (467)
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<222> (476)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (481)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (483)..(484)
<223> n equals a, t, g, or c

<220>
<221> misc_feature
<222> (494)
<223> n equals a, t, g, or c

<400> 9
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tctttgaacc accagctcca ggagaaggca actccagtca gaacagcaga aataagcgtg 120
ccggttcaggg tccagaagaa acagtcaactc aagactgtt gcaactgntt gcagacagtg 180
aaacaccaac tataaaaaaa ggctcccttc tgntgccaca tttggggccaa ggaatggaga 240
gatttcttcg tctggaaaca ttttgccaaa ctcttcagat actctttntct ctctggaaat 300
caaaggaaaa tctctactta gattnacaca tttgtccca tgggtntctt aagttttaaa 360
aggggagtgc ccttaggagg aaaaggggat aaatattggc caaggnactg gttanttnt 420
aaatatggtc aggttntat anctggtagg cctcgccatg ggcatttnatt cangggagg 480
ncnntctttt gggntga                                         497

<210> 10
<211> 27
<212> DNA
<213> Homo sapiens

<220>
<223> Neutrokinin-alpha forward primer containing BamHI

    restriction site

<400> 10
gtgggatcca gcctccgggc agagctg

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<210> 11
<211> 33
<212> DNA
<213> Homo sapiens

<220>
<223> Neutrokinine-alpha reverse primer containing HindIII restriction site and sequence complementary to two stop codons

<400> 11
gtgaagctt tattacagca gtttcaatgc acc 33

<210> 12
<211> 26
<212> DNA
<213> Homo sapiens

<220>
<223> Neutrokinine-alpha forward primer containing BspHI restriction site

<400> 12
gtgtcatgag cctccgggca gagctg 26

<210> 13
<211> 33
<212> DNA
<213> Homo sapiens

<220>
<223> Neutrokinine-alpha reverse primer containing HindIII restriction site and sequence complementary to two stop codons

<400> 13
gtgaagctt tattacagca gtttcaatgc acc 33

<210> 14
<211> 28
<212> DNA
<213> Homo sapiens

<220>
<223> Neutrokinine-alpha forward primer containing BamHI restriction site

<400> 14
gtgggatccc cgggcagagc tgcaggc 28

<210> 15
<211> 33
<212> DNA
<213> Homo sapiens

<220>
 <223> Neutrokinine-alpha reverse primer containing BamHI restriction site and sequence complementary to two stop codons

<400> 15
 gtgggatcct tattacagca gtttcaatgc acc

33

<210> 16
 <211> 129
 <212> DNA
 <213> Homo sapiens

<220>
 <223> Neutrokinine-alpha forward primer containing a Bam HI restriction site, Kozak sequence, AUG start codon, and sequence encoding the secretory leader peptide of human IL-6 gene

<400> 16
 gcgggatccg ccaccatgaa ctccttctcc acaagcgcct tcggtccagt tgccttctcc 60
 ctggggctgc tcctgggttt gcctgctgcc ttccctgccc cagttgtgag acaaggggac 120
 ctggccagc 129

<210> 17
 <211> 30
 <212> DNA
 <213> Homo sapiens

<220>
 <223> Neutrokinine-alpha reverse primer containing BamHI restriction site

<400> 17
 gtgggatcct tacagcagtt tcaatgcacc 30

<210> 18
 <211> 903
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)..(798)

<400> 18
 atg gat gac tcc aca gaa agg gag cag tca cgc ctt act tct tgc ctt 48
 Met Asp Asp Ser Thr Glu Arg Glu Gln Ser Arg Leu Thr Ser Cys Leu
 1 5 10 15

aag aaa aga gaa gaa atg aaa ctg aag gag tgt gtt tcc atc ctc cca 96
 Lys Lys Arg Glu Glu Met Lys Leu Lys Glu Cys Val Ser Ile Leu Pro
 20 25 30

cgg aag gaa agc ccc tct gtc cga tcc tcc aaa gac gga aag ctg ctg 144

Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly Lys Leu Leu			
35	40	45	
gct gca acc ttg ctg ctg gca ctg ctg tct tgc tgc ctc acg gtg gtg		192	
Ala Ala Thr Leu Leu Ala Leu Leu Ser Cys Cys Leu Thr Val Val			
50	55	60	
tct ttc tac cag gtg gcc gcc ctg caa ggg gac ctg gcc agc ctc cgg		240	
Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp Leu Ala Ser Leu Arg			
65	70	75	80
gca gag ctg cag ggc cac cac gcg gag aag ctg cca gca gga gca gga		288	
Ala Glu Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Gly Ala Gly			
85	90	95	
gcc ccc aag gcc ggc ctg gag gaa gct cca gct gtc acc gcg gga ctg		336	
Ala Pro Lys Ala Gly Leu Glu Glu Ala Pro Ala Val Thr Ala Gly Leu			
100	105	110	
aaa atc ttt gaa cca cca gct cca gga gaa ggc aac tcc agt cag aac		384	
Lys Ile Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Asn			
115	120	125	
agc aga aat aag cgt gcc gtt cag ggt cca gaa gaa aca gga tct tac		432	
Ser Arg Asn Lys Arg Ala Val Gln Gly Pro Glu Glu Thr Gly Ser Tyr			
130	135	140	
aca ttt gtt cca tgg ctt ctc agc ttt aaa agg gga agt gcc cta gaa		480	
Thr Phe Val Pro Trp Ile Leu Ser Phe Lys Arg Gly Ser Ala Leu Glu			
145	150	155	160
gaa aaa gag aat aaa ata ttg gtc aaa gaa act ggt tac ttt ttt ata		528	
Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr Phe Phe Ile			
165	170	175	
tat ggt cag gtt tta tat act gat aag acc tac gcc atg gga cat cta		576	
Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met Gly His Leu			
180	185	190	
att cag agg aag gtc cat gtc ttt ggg gat gaa ttg agt ctg gtg		624	
Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser Leu Val			
195	200	205	
act ttg ttt cga tgt att caa aat atg cct gaa aca cta ccc aat aat		672	
Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu Pro Asn Asn			
210	215	220	
tcc tgc tat tca gct ggc att gca aaa ctg gaa gaa gga gat gaa ctc		720	
Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu Leu			
225	230	235	240
caa ctt gca ata cca aga gaa aat gca caa ata tca ctg gat gga gat		768	
Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu Asp Gly Asp			
245	250	255	
gtc aca ttt ttt ggt gca ttg aaa ctg ctg tgacctactt acaccatgtc		818	
Val Thr Phe Phe Gly Ala Leu Lys Leu Leu			
260	265		
tgttagctatt ttcctccctt tctctgtacc tctaagaaga aagaatctaa ctgaaaatac		878	

aaaaaaaaaa aaaaaaaaaaa aaaaa

903

<210> 19
 <211> 266
 <212> PRT
 <213> Homo sapiens

<400> 19
 Met Asp Asp Ser Thr Glu Arg Glu Gln Ser Arg Leu Thr Ser Cys Leu
 1 5 10 15
 Lys Lys Arg Glu Glu Met Lys Leu Lys Glu Cys Val Ser Ile Leu Pro
 20 25 30
 Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly Lys Leu Leu
 35 40 45
 Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Cys Cys Leu Thr Val Val
 50 55 60
 Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp Leu Ala Ser Leu Arg
 65 70 75 80
 Ala Glu Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Gly Ala Gly
 85 90 95
 Ala Pro Lys Ala Gly Leu Glu Glu Ala Pro Ala Val Thr Ala Gly Leu
 100 105 110
 Lys Ile Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Asn
 115 120 125
 Ser Arg Asn Lys Arg Ala Val Gln Gly Pro Glu Glu Thr Gly Ser Tyr
 130 135 140
 Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser Ala Leu Glu
 145 150 155 160
 Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr Phe Phe Ile
 165 170 175
 Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met Gly His Leu
 180 185 190
 Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser Leu Val
 195 200 205
 Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu Pro Asn Asn
 210 215 220
 Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu Leu
 225 230 235 240
 Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu Asp Gly Asp
 245 250 255
 Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
 260 265

<210> 20
<211> 136

<212> PRT
<213> Homo sapiens

<400> 20
His Ser Val Leu His Leu Val Pro Ile Asn Ala Thr Ser Lys Asp Asp
1 5 10 15
Ser Asp Val Thr Glu Val Met Trp Gln Pro Ala Leu Arg Arg Gly Arg
20 25 30
Gly Leu Gln Ala Gln Gly Tyr Gly Val Arg Ile Gln Asp Ala Gly Val
35 40 45
Tyr Leu Leu Tyr Ser Gln Val Leu Phe Gln Asp Val Thr Phe Thr Met
50 55 60
Gly Gln Val Val Ser Arg Glu Gly Gln Gly Arg Gln Glu Thr Leu Phe
65 70 75 80
Arg Cys Ile Arg Ser Met Pro Ser His Pro Asp Arg Ala Tyr Asn Ser
85 90 95
Cys Tyr Ser Ala Gly Val Phe His Leu His Gln Gly Asp Ile Leu Ser
100 105 110
Val Ile Pro Arg Ala Arg Ala Lys Leu Asn Leu Ser Pro His Gly
115 120 125
Thr Phe Leu Gly Phe Val Lys Leu
130 135

<210> 21
<211> 462
<212> DNA
<213> Homo sapiens

<400> 21
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tctgaaactc cgaccatcca gaaagggttct tacacctttg ttcccttggct gctttcttc 120
aaacgtggtt ctgccttggaa agagaaaagaa aacaaaatcc tggtaaaga aactggttac 180
ttctttatct acggtcaggt tctttacact gataagacct acggccatggg tcacctgatt 240
cagcgtaaaga aagtcacgt ttccgggtgac gagctgtctc tggtaactct gtttcgtgc 300
attcagaaca tgccggaaac tcttcctaact aactccgtct actctgtctgg catcgaaaa 360
ctggaagagg gtgtatgaact gcagctggca attcctcggt aaaacgcaca aatttctctg 420
gacggtgatg taaccttctt tggtgactg aaacttctgt aa 462

<210> 22
<211> 1040
<212> DNA
<213> Mus musculus

<220>

<221> CDS

<222> (1)..(468)

<400> 22

cgc	gtg	gta	gac	ctc	tca	gct	cct	cct	gca	cca	tgc	ctg	cct	gga	tgc	48
Arg	Val	Val	Asp	Leu	Ser	Ala	Pro	Pro	Ala	Pro	Cys	Leu	Pro	Gly	Cys	
1				5					10				15			

cgc	cat	tct	caa	cat	gat	aat	gga	atg	aac	ctc	aga	aac	aga	act	96	
Arg	His	Ser	Gln	His	Asp	Asp	Asn	Gly	Met	Asn	Leu	Arg	Asn	Arg	Thr	
20					25					30						

tac	aca	ttt	gtt	cca	tgg	ctt	ctc	agc	ttt	aaa	aga	gga	aat	gcc	ttg	144
Tyr	Thr	Phe	Val	Pro	Trp	Leu	Leu	Ser	Phe	Lys	Arg	Gly	Asn	Ala	Leu	
35					40					45						

gag	gag	aaa	gag	aac	aaa	ata	gtg	gtg	agg	caa	aca	ggc	tat	ttc	ttc	192
Glu	Glu	Lys	Glu	Asn	Lys	Ile	Val	Val	Arg	Gln	Thr	Gly	Tyr	Phe	Phe	
50				55					60							

atc	tac	agc	cag	gtt	cta	tac	acg	gac	ccc	atc	ttt	gct	atg	ggt	cat	240
Ile	Tyr	Ser	Gln	Val	Leu	Tyr	Thr	Asp	Pro	Ile	Phe	Ala	Met	Gly	His	
65				70					75			80				

gtc	atc	cag	agg	aag	aaa	gta	cac	gtc	ttt	ggg	gac	gag	ctg	agc	ctg	288
Val	Ile	Gln	Arg	Lys	Lys	Val	His	Val	Phe	Gly	Asp	Glu	Leu	Ser	Leu	
85					90					95						

gtg	acc	ctg	ttc	cga	tgt	att	cag	aat	atg	ccc	aaa	aca	ctg	ccc	aac	336
Val	Thr	Leu	Phe	Arg	Cys	Ile	Gln	Asn	Met	Pro	Lys	Thr	Leu	Pro	Asn	
100					105					110						

aat	tcc	tgc	tac	tcg	gct	ggc	atc	gcg	agg	ctg	gaa	gaa	gga	gat	gag	384
Asn	Ser	Cys	Tyr	Ser	Ala	Gly	Ile	Ala	Arg	Leu	Glu	Gly	Asp	Glu		
115					120					125						

att	cag	ctt	gca	att	cct	cg	gag	aat	gca	cag	att	tca	cgc	aac	gga	432
Ile	Gln	Leu	Ala	Ile	Pro	Arg	Glu	Asn	Ala	Gln	Ile	Ser	Arg	Asn	Gly	
130					135					140						

gac	gac	acc	ttc	ttt	ggt	gcc	cta	aaa	ctg	ctg	taa	ctcacttgct		478	
Asp	Asp	Thr	Phe	Phe	Gly	Ala	Leu	Lys	Leu	Leu					
145					150					155					

ggagtgcgtg atccccttcc ctcgtttct ctgtacccctc gagggagaaa cagacgactg 538

aaaaaaactaa aagatgggaa aagccgtcag cgaaagtttt ctcgtgaccc gttgaatctg 598

atccaaacca ggaaatataa cagacagcca caaccgaagt gtgccatgtg agttatgaga 658

aacggagccc gcgctcagaa agaccggatg aggaagaccg ttttctccag tcctttgcca 718

acacgcaccc caacccttgct ttttgccttg ggtgacacat gttcagaatg cagggagatt 778

tccttgtttt gcgatttgcc atgagaagag ggcccacaac tgcaggtcac tgaagcattc 838

acgctaagtc tcaggattta ctctcccttc tcatgctaag tacacacacg ctctttcca 898

ggtaatacta tggataacta tggaaaggtt gtttggggaa aatcttagaa gtcttgaact 958

ggcaatagac aaaaatcctt ataaattcaa gtgtaaaata aacttaatta aaaaggttta 1018
 agtgtgaaaa aaaaaaaaaaa aa 1040

<210> 23
 <211> 155
 <212> PRT
 <213> Mus musculus

<400> 23
 Arg Val Val Asp Leu Ser Ala Pro Pro Ala Pro Cys Leu Pro Gly Cys
 1 5 10 15
 Arg His Ser Gln His Asp Asp Asn Gly Met Asn Leu Arg Asn Arg Thr
 20 25 30
 Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Asn Ala Leu
 35 40 45
 Glu Glu Lys Glu Asn Lys Ile Val Val Arg Gln Thr Gly Tyr Phe Phe
 50 55 60
 Ile Tyr Ser Gln Val Leu Tyr Thr Asp Pro Ile Phe Ala Met Gly His
 65 70 75 80
 Val Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser Leu
 85 90 95
 Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Lys Thr Leu Pro Asn
 100 105 110
 Asn Ser Cys Tyr Ser Ala Gly Ile Ala Arg Leu Glu Glu Gly Asp Glu
 115 120 125
 Ile Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly
 130 135 140
 Asp Asp Thr Phe Phe Gly Ala Leu Lys Leu Leu
 145 150 155

<210> 24
 <211> 26
 <212> DNA
 <213> Homo sapiens

<400> 24
 ccaccagctc caggagaagg caactc 26

<210> 25
 <211> 19
 <212> DNA
 <213> Homo sapiens

<400> 25
 accgcgggac tgaaaatct 19

<210> 26
 <211> 23
 <212> DNA
 <213> Homo sapiens

<400> 26

cacgcttatt tctgctgttc tga

23

<210> 27
 <211> 657
 <212> DNA
 <213> Macaca irus

<400> 27
 taccagggtgg cggcggtgca aggggacctg gccagcctcc gggcagagct gcagggccac 60
 cacgcggaga agctgccagc aagagcaaga gcccccaagg ccggctctggg ggaagctcca 120
 gctgtcaccg caggactgaa aatcttgcaccc ccaccagctc caggagaagg caactccagt 180
 cagagcagca gaaataagcg tgctattcag ggtgcagaag aacacgtcat tcaagactgc 240
 ttgcaactga ttgcagacag tgaaacacca actatacataa aaggatctta cacattgtt 300
 ccatggcttc tcagcttaa aaggggaaagt gcccctagaag aaaaagagaa taaaatattg 360
 gtcaaaagaaa ctggttactt ttttatataat ggtcaggattt tataactga taagacctat 420
 gccatgggac atctaattca gaggaaaaaa gtccatgtct ttggggatga attgagtctg 480
 gtgactttgt ttgcgttat tcaaaatatg cctgaaacac tacccaaataa ttcctgctat 540
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 aatgcacaaa tatcactgga tggagatgtc acatttttg gtgcctcaa actgctg 657

<210> 28
 <211> 219
 <212> PRT
 <213> Macaca irus

<400> 28
 Tyr Gln Val Ala Ala Val Gln Gly Asp Leu Ala Ser Leu Arg Ala Glu
 1 5 10 15

Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Arg Ala Arg Ala Pro
 20 25 30

Lys Ala Gly Leu Gly Glu Ala Pro Ala Val Thr Ala Gly Leu Lys Ile
 35 40 45

Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Ser Ser Arg
 50 55 60

Asn Lys Arg Ala Ile Gln Gly Ala Glu Glu Thr Val Ile Gln Asp Cys
 65 70 75 80

Leu Gln Leu Ile Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys Gly Ser
 85 90 95

Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser Ala Leu
 100 105 110

Glu Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr Phe Phe
 115 120 125

Ile Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met Gly His
 130 135 140

Leu Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser Leu
 145 150 155 160

Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu Pro Asn
 165 170 175

Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu
 180 185 190

Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu Asp Gly
 195 200 205

Asp Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
 210 215

<210> 29

<211> 657

<212> DNA

<213> Macaca mulatta (Rhesus Monkey)

<400> 29

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 cacgcggaga agctgccagc aagagcaaga gcccccaagg cgggtctggg ggaagctcca 120
 gctgtcaccg cgggactgaa aatctttgaa ccaccagctc caggagaagg caactccagt 180
 cagagcagca gaaataagcg tgctattcag ggtgcagaag aaacagtcat tcaagactgc 240
 ttgcaactga ttgcagacag taaaacacca actatacaca aaggatctta cacattgtt 300
 ccatggcttc tcagcttaa aaggggaaat gcccataaag aaaaagagaa taaaatattg 360
 gtcaaaagaaa ctggttactt ttttatatat ggtcagggtt tatacactga taagacctat 420
 gccatggac atctaattca gaggaaaaaa gtccatgtct ttggggatga attgagtctg 480
 gtgactttgt ttcgatgtat tcaaaatatg cctgaaacac tacccaataa ttcctgctat 540
 tcagctgca ttgcaaaaact ggaagaaggg gatgaacttc aacttgcaat accacgagaa 600
 aatgcacaaa tatcaactgga tggagatgtc acatttttg gtgcctcaa actgctg 657

<210> 30

<211> 219

<212> PRT

<213> Macaca mulatta (Rhesus Monkey)

<400> 30

Tyr Gln Val Ala Ala Val Gln Gly Asp Leu Ala Ser Leu Arg Ala Glu
 1 5 10 15

Leu Gln Ser His His Ala Glu Lys Leu Pro Ala Arg Ala Arg Ala Pro
 20 25 30

Lys Ala Gly Leu Gly Glu Ala Pro Ala Val Thr Ala Gly Leu Lys Ile
 35 40 45

Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Ser Ser Arg
 50 55 60

Asn Lys Arg Ala Ile Gln Gly Ala Glu Glu Thr Val Ile Gln Asp Cys
 65 70 75 80

Leu Gln Leu Ile Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys Gly Ser
 85 90 95

Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser Ala Leu
 100 105 110

Glu Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr Phe Phe
 115 120 125

Ile Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met Gly His
 130 135 140

Leu Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser Leu
 145 150 155 160

Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu Pro Asn
 165 170 175

Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu
 180 185 190

Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu Asp Gly
 195 200 205

Asp Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
 210 215

<210> 31

<211> 38

<212> DNA

<213> Homo sapiens

<220>

<223> Neutrokinine-alpha forward primer containing sequence
 encoding PSC signal peptide C-terminus

<400> 31

ggtcgcgtt tctaacgcgg ccgttcaggg tccagaag

38

<210> 32

<211> 49

<212> DNA

<213> Homo sapiens

<220>

<223> Reverse primer for amplifying Neutrokinine-alpha containing
 reverse complement sequence of the pA2GP vector and Kpn I
 restriction site

<400> 32

ctggttcggc ccaaggtacc aagcttgtac ctttagatctt ttctagatc

49

<210> 33

<211> 21

<212> DNA

<213> Homo sapiens

<220>

<223> forward primer that anneals to PSC baculovirus transfer
 plasmid pMGS12

<400> 33

ctggtagttc ttccggagtgt g

21

<210> 34
<211> 19
<212> DNA
<213> Homo sapiens

<220>
<223> reverse primer that anneals to PSC baculovirus transfer
plasmid pMGS12

<400> 34
cgcgttagaa acggcgacc

19

<210> 35
<211> 22
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (7)
<223> n equals deoxyinosine or dideoxyinosine

<220>
<221> misc_feature
<222> (12)
<223> n equals deoxyinosine or dideoxyinosine

<220>
<221> misc_feature
<222> (16)
<223> n equals deoxyinosine or dideoxyinosine

<220>
<223> Neutrokinin-alpha degenerate oligonucleotide forward
primer

<400> 35
taccagntgg cngccntgca ag

22

<210> 36
<211> 22
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<222> (3)
<223> n equals deoxyinosine or dideoxyinosine

<220>
<221> misc_feature
<222> (14)
<223> n equals deoxyinosine or dideoxyinosine

<220>
<221> misc_feature
<222> (16)..(17)
<223> n equals deoxyinosine or dideoxyinosine

<220>
<223> Neutrokinin-alpha degenerate oligonucleotide reverse
primer

<400> 36
gtnacagcag tttnanngca cc

22

<210> 37
<211> 867
<212> DNA
<213> *Mus musculus*

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<400> 37
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gaagatatga aagtgggata tgatcccattt actccgcaga aggaggaggg tgccctggtt 120
gggatctgca gggatggaaag gctgctggct gctaccctcc tgctggccct gttgtccagc 180
agtttcacag cgatgtcctt gtaccagttg gctgccttgc aagcagaccc gatgaacctg 240
cgcatggagc tgcaagagcta ccgagggttca gcaacaccag ccggccgggg tgctccagag 300
ttgaccgctg gagtcaaact cctgacacccg gcagctcctc gaccccacaa ctccagccgc 360
ggccacagga acagacgcgc ctccagggc ccagaggaaa cagaacaaga tgtagaccc 420
tcagctcctc ctgcaccatg cctgccttggc tgccgcccattt ctcaacatga tgataatggc 480
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gccttggagg agaaaagagaaa caaaatagtg gtgaggcaaa caggctattt ctccatctac 600
agccagggttc tatacacccgga ccccatcttt gctatgggtc atgtcatcca gaggaaagaaa 660
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cccaaaacac tgcccaacaa ttccctgtac tcggctggca tcgcgaggct ggaagaagga 780
gatgagattc agcttgcata tcctcgggag aatgcacaga tttcacgcaa cggagacgac 840
accttctttg gtggccctaaa actgctg 867

<210> 38
<211> 289
<212> PRT
<213> *Mus musculus*

<400> 38
Met Asp Glu Ser Ala Lys Thr Leu Pro Pro Pro Cys Leu Cys Phe Cys
1 5 10 15

Ser Glu Lys Gly Glu Asp Met Lys Val Gly Tyr Asp Pro Ile Thr Pro
20 25 30

Gln Lys Glu Glu Gly Ala Trp Phe Gly Ile Cys Arg Asp Gly Arg Leu
 35 40 45

Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Ser Ser Phe Thr Ala
50 55 60

Met Ser Leu Tyr Gln Leu Ala Ala Leu Gln Ala Asp Leu Met Asn Leu
65 70 75 80

Arg Met Glu Leu Gln Ser Tyr Arg Gly Ser Ala Thr Pro Ala Ala Ala
85 90 95

Gly Ala Pro Glu Leu Thr Ala Gly Val Lys Leu Leu Thr Pro Ala Ala
100 105 110

Pro Arg Pro His Asn Ser Ser Arg Gly His Arg Asn Arg Arg Ala Phe

115	120	125
Gln Gly Pro Glu Glu Thr Glu Gln Asp Val Asp Leu Ser Ala Pro Pro		
130	135	140
Ala Pro Cys Leu Pro Gly Cys Arg His Ser Gln His Asp Asp Asn Gly		
145	150	155
Met Asn Leu Arg Asn Ile Ile Gln Asp Cys Leu Gln Leu Ile Ala Asp		
165	170	175
Ser Asp Thr Pro Ala Leu Glu Glu Lys Glu Asn Lys Ile Val Val Arg		
180	185	190
Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu Tyr Thr Asp Pro		
195	200	205
Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys Val His Val Phe		
210	215	220
Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met		
225	230	235
Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Arg		
245	250	255
Leu Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro Arg Glu Asn Ala		
260	265	270
Gln Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly Ala Leu Lys Leu		
275	280	285

Leu

<210> 39			
<211> 309			
<212> PRT			
<213> Mus musculus			
<400> 39			
Met Asp Glu Ser Ala Lys Thr Leu Pro Pro Pro Cys Leu Cys Phe Cys			
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Ser Glu Lys Gly Glu Asp Met Lys Val Gly Tyr Asp Pro Ile Thr Pro			
20	25	30	
Gln Lys Glu Glu Gly Ala Trp Phe Gly Ile Cys Arg Asp Gly Arg Leu			
35	40	45	
Leu Ala Ala Thr Leu Leu Ala Leu Leu Ser Ser Ser Phe Thr Ala			
50	55	60	
Met Ser Leu Tyr Gln Leu Ala Ala Leu Gln Ala Asp Leu Met Asn Leu			
65	70	75	80
Arg Met Glu Leu Gln Ser Tyr Arg Gly Ser Ala Thr Pro Ala Ala Ala			
85	90	95	

Gly Ala Pro Glu Leu Thr Ala Gly Val Lys Leu Leu Thr Pro Ala Ala
 100 105 110
 Pro Arg Pro His Asn Ser Ser Arg Gly His Arg Asn Arg Arg Ala Phe
 115 120 125
 Gln Gly Pro Glu Glu Thr Glu Gln Asp Val Asp Leu Ser Ala Pro Pro
 130 135 140
 Ala Pro Cys Leu Pro Gly Cys Arg His Ser Gln His Asp Asp Asn Gly
 145 150 155 160
 Met Asn Leu Arg Asn Ile Ile Gln Asp Cys Leu Gln Leu Ile Ala Asp
 165 170 175
 Ser Asp Thr Pro Thr Ile Arg Lys Gly Thr Tyr Thr Phe Val Pro Trp
 180 185 190
 Leu Leu Ser Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys
 195 200 205
 Ile Val Val Arg Gln Thr Gly Tyr Phe Ile Tyr Ser Gln Val Leu
 210 215 220
 Tyr Thr Asp Pro Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys
 225 230 235 240
 Val His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys
 245 250 255
 Ile Gln Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala
 260 265 270
 Gly Ile Ala Arg Leu Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro
 275 280 285
 Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly
 290 295 300
 Ala Leu Lys Leu Leu
 305

<210> 40
 <211> 290
 <212> PRT
 <213> Mus musculus

<400> 40
 Met Asp Glu Ser Ala Lys Thr Leu Pro Pro Pro Cys Leu Cys Phe Cys
 1 5 10 15
 Ser Glu Lys Gly Glu Asp Met Lys Val Gly Tyr Asp Pro Ile Thr Pro
 20 25 30
 Gln Lys Glu Glu Gly Ala Trp Phe Gly Ile Cys Arg Asp Gly Arg Leu
 35 40 45
 Leu Ala Ala Thr Leu Leu Ala Leu Leu Ser Ser Ser Phe Thr Ala

50	55	60
Met Ser Leu Tyr Gln Leu Ala Ala Leu Gln Ala Asp Leu Met Asn Leu		
65	70	75
Arg Met Glu Leu Gln Ser Tyr Arg Gly Ser Ala Thr Pro Ala Ala Ala		
85	90	95
Gly Ala Pro Glu Leu Thr Ala Gly Val Lys Leu Leu Thr Pro Ala Ala		
100	105	110
Pro Arg Pro His Asn Ser Ser Arg Gly His Arg Asn Arg Arg Ala Phe		
115	120	125
Gln Gly Pro Glu Glu Thr Glu Gln Asp Val Asp Leu Ser Ala Pro Pro		
130	135	140
Ala Pro Cys Leu Pro Gly Cys Arg His Ser Gln His Asp Asp Asn Gly		
145	150	155
Met Asn Leu Arg Asn Arg Thr Tyr Thr Phe Val Pro Trp Leu Leu Ser		
165	170	175
Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys Ile Val Val		
180	185	190
Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu Tyr Thr Asp		
195	200	205
Pro Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys Val His Val		
210	215	220
Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn		
225	230	235
Met Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala		
245	250	255
Arg Leu Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro Arg Glu Asn		
260	265	270
Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly Ala Leu Lys		
275	280	285
Leu Leu		
290		

<210> 41
 <211> 152
 <212> PRT
 <213> Rattus sp

<400> 41
 Ala Phe Gln Gly Pro Glu Glu Thr Val Ile Gln Asp Cys Leu Gln Leu
 1 5 10 15
 Ile Ala Asp Ser Asn Thr Pro Thr Ile Arg Lys Gly Thr Tyr Thr Phe
 20 25 30

Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys
 35 40 45

Glu Asn Lys Ile Val Val Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser
 50 55 60

Gln Val Leu Tyr Thr Asp Pro Ile Phe Ala Met Gly His Val Ile Gln
 65 70 75 80

Arg Lys Lys Ile His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu
 85 90 95

Phe Arg Cys Ile Gln Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys
 100 105 110

Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu Val Gln Leu
 115 120 125

Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr
 130 135 140

Phe Phe Gly Ala Leu Lys Leu Leu
 145 150

<210> 42
 <211> 165
 <212> PRT
 <213> Rattus sp

<400> 42
 Ala Phe Gln Gly Pro Glu Glu Thr Glu Gln Asp Val Asp Leu Ser Ala
 1 5 10 15

Thr Pro Val Pro Ser Leu Pro Gly Asn Cys His Ala Ser His His Asp
 20 25 30

Glu Asn Gly Leu Asn Leu Arg Thr Arg Thr Tyr Thr Phe Val Pro Trp
 35 40 45

Leu Leu Ser Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys
 50 55 60

Ile Val Val Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu
 65 70 75 80

Tyr Thr Asp Pro Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys
 85 90 95

Ile His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys
 100 105 110

Ile Gln Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala
 115 120 125

Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro
 130 135 140

Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly

145	150	155	160
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Ala Leu Lys Leu Leu
165

<210> 43
<211> 184
<212> PRT
<213> Rattus sp

<400> 43
Ala Phe Gln Gly Pro Glu Glu Thr Glu Gln Asp Val Asp Leu Ser Ala
1 5 10 15

Thr Pro Ala Pro Ser Leu Pro Gly Asn Cys His Ala Ser His His Asp
20 25 30

Glu Asn Gly Leu Asn Leu Arg Thr Ile Ile Gln Asp Cys Leu Gln Leu
35 40 45

Ile Ala Asp Ser Asn Thr Pro Thr Ile Arg Lys Gly Thr Tyr Thr Phe
50 55 60

Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys
65 70 75 80

Glu Asn Lys Ile Val Val Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser
85 90 95

Gln Val Leu Tyr Thr Asp Pro Ile Phe Ala Met Gly His Val Ile Gln
100 105 110

Arg Lys Lys Ile His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu
115 120 125

Phe Arg Cys Ile Gln Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys
130 135 140

Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu Ile Gln Leu
145 150 155 160

Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Arg Asn Gln Asp Asp Thr
165 170 175

Phe Phe Gly Ala Leu Lys Leu Leu
180

<210> 44
<211> 133
<212> PRT
<213> Rattus sp

<400> 44
Ala Phe Gln Gly Pro Glu Glu Thr Gly Thr Tyr Thr Phe Val Pro Trp
1 5 10 15

Leu Leu Ser Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys
20 25 30

Ile Val Val Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu
35 40 45

Tyr Thr Asp Pro Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys
50 55 60

Ile His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys
65 70 75 80

Ile Gln Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala
85 90 95

Gly Ile Ala Lys Leu Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro
100 105 110

Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly
115 120 125

Ala Leu Lys Leu Leu
130

<210> 45

<211> 17

<212> PRT

<213> Homo sapiens

<400> 45

Met Leu Gln Asn Ser Ala Val Leu Leu Leu Val Ile Ser Ala Ser
1 5 10 15

Ala

<210> 46

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<221> SIGNAL

<222> (1)..(22)

<223> consensus signal sequence

<400> 46

Met Pro Thr Trp Ala Trp Trp Leu Phe Leu Val Leu Leu Ala Leu
1 5 10 15

Trp Ala Pro Ala Arg Gly
20

<210> 47

<211> 250

<212> PRT

<213> Homo sapiens

<400> 47

Met Pro Ala Ser Ser Pro Phe Leu Leu Ala Pro Lys Gly Pro Pro Gly
 1 5 10 15

Asn Met Gly Gly Pro Val Arg Glu Pro Ala Leu Ser Val Ala Leu Trp
 20 25 30

Leu Ser Trp Gly Ala Ala Leu Gly Ala Val Ala Cys Ala Met Ala Leu
 35 40 45

Leu Thr Gln Gln Thr Glu Leu Gln Ser Leu Arg Arg Glu Val Ser Arg
 50 55 60

Leu Gln Gly Thr Gly Gly Pro Ser Gln Asn Gly Glu Gly Tyr Pro Trp
 65 70 75 80

Gln Ser Leu Pro Glu Gln Ser Ser Asp Ala Leu Glu Ala Trp Glu Asn
 85 90 95

Gly Glu Arg Ser Arg Lys Arg Arg Ala Val Leu Thr Gln Lys Gln Lys
 100 105 110

Lys Gln His Ser Val Leu His Leu Val Pro Ile Asn Ala Thr Ser Lys
 115 120 125

Asp Asp Ser Asp Val Thr Glu Val Met Trp Gln Pro Ala Leu Arg Arg
 130 135 140

Gly Arg Gly Leu Gln Ala Gln Gly Tyr Gly Val Arg Ile Gln Asp Ala
 145 150 155 160

Gly Val Tyr Leu Leu Tyr Ser Gln Val Leu Phe Gln Asp Val Thr Phe
 165 170 175

Thr Met Gly Gln Val Val Ser Arg Glu Gly Gln Gly Arg Gln Glu Thr
 180 185 190

Leu Phe Arg Cys Ile Arg Ser Met Pro Ser His Pro Asp Arg Ala Tyr
 195 200 205

Asn Ser Cys Tyr Ser Ala Gly Val Phe His Leu His Gln Gly Asp Ile
 210 215 220

Leu Ser Val Ile Ile Pro Arg Ala Arg Ala Lys Leu Asn Leu Ser Pro
 225 230 235 240

His Gly Thr Phe Leu Gly Phe Val Lys Leu
 245 250

<210> 48
 <211> 38
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotide primer

<400> 48
 cagactggat cggccaccat ggatgactcc acagaaag

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<210> 49
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide Primer

<400> 49
cagactggta ccgtcctgcg tgcactacat ggc 33

<210> 50
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide Primer

<400> 50
tggtgtcttt ctaccaggta g 21

<210> 51
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 51
tttcttctgg accctgaacg g 21

<210> 52
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<212> DNA
<213> Artificial

<220>
<223> pML124

<400> 52
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agctttggag attatcgtca ctgcaatgct tcgcaatatg ggcggaaatg accaacagcg 180
gttgattgat caggttagagg gggcgctgta cgaggtaaag cccgatgcca gcattcctga 240
cgacgatacg gagctgctgc gcgattacgt aaagaagtta ttgaagcatc ctcgtcagta 300
aaaagttaat cttttcaaca gctgtcataa agttgtcactg gccgagactt atagtcgctt 360
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atcgccgaca	tcaccgatgg	ggaagatcgg	gctcgccact	tcgggctcat	gagcgcttgt	600
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ggagaactgt	aatgcgcaa	accaaccctt	ggcagaacat	atccatcg	tccggcatct	1500
ccagcagccg	cacgcggcgc	atctcg	gcgttgg	ctggccacgg	gtgcgc	1560
tcgtgctcct	gtcg	accggct	gctgg	ttgc	ttact	1620
atgaatcacc	gatacgcgag	cgaacgtgaa	gcgactgt	ctgcaaaacg	tctgcgac	1680
gagcaacaac	atgaatggc	tgcgtt	gtgtt	cgta	aagtctggaa	1740
cagcgccctg	caccattatg	ttccggatct	gcatcg	cagg	atgctgt	1800
gaacacccat	atctgttata	acgaagcg	ggcattgacc	ctgagt	gatt	1860
cccgccgc	ccataccg	agttgttac	cctcaca	ttccag	taac	1920
catcatcagt	aaccgtatc	gtgagcatcc	tctctcg	ttt	catcggtatc	1980
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<220>
 <223> MBP signal sequence

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 <212> PRT
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<220>
 <223> MBPss-Neutrokinin-alpha

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 35 40 45
 Pro Thr Ile Gln Lys Gly Ser Tyr Thr Phe Val Pro Trp Leu Leu Ser
 50 55 60

Phe Lys Arg Gly Ser Ala Leu Glu Glu Lys Glu Asn Lys Ile Leu Val
 65 70 75 80

Lys Glu Thr Gly Tyr Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr Asp
 85 90 95

Lys Thr Tyr Ala Met Gly His Leu Ile Gln Arg Lys Lys Val His Val
 100 105 110

Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn
115 120 125

Met Pro Glu Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala
130 135 140

Lys Leu Glu Glu Gly Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu Asn
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Ala Gln Ile Ser Leu Asp Gly Asp Val Thr Phe Phe Gly Ala Leu Lys
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Leu Leu

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<220>
<223> wild-type Shine Dalgarno box and its adjacent sequence

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21

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21